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CONTENTS

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Attempts to Induce Stomach Tumors

IV. The Effects of (a) Cholesteryl Esters Heated to 300° C., and (b) Cholesterol Heated to 430° C.*

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The frequency of human gastric carcinoma contrasts sharply with the rarity of this lesion in animals, and the many unsuccessful attempts to induce malignant stomach tumors in experimental animals have been reviewed by several authors (16, 18, 34). Stewart and Lorenz (32) obtained transplantable adenocarcinoma in mice of the C3H, I, and C57 brown strains by injecting dispersions of 20-methylcholanthrene into the wall of the glandular stomach, but apart from this only squamous carcinoma of the forestomach has been induced (5, 19, 37), although papillomas of at least two types (25) have been elicited by several methods (16) in rats and mice.

Roffo's assertion (26-29) that he had produced adenocarcinoma of the glandular stomach in rats fed on either fats or cholesterol heated to 350° C. for half an hour raised hopes that a method had been found for obtaining stomach tumors comparable to those found in human beings; moreover, the method was not unlike procedures that are common enough in ordinary human gastronomics. Roffo's results, however, were adversely criticized by Klein and Palmer (18), who pointed out that his criteria of malignancy were not sufficiently rigid. Furthermore, attempts to repeat his heated-fat experiments have not led to any adenocarcinoma. Beck and Peacock (2) found frequent gastro-papillomatosis of the forestomach without lesions of the glandular zone in rats, while recently Morris, Larsen, and Lippincott (22) reported only occasional chronic gastric ulcers in rats receiving diets containing up to 50 per cent lard that had been heated to 350° C. for half an hour.

Roffo's statement that rats fed on cholesterol that had been heated to 350° C. for half an hour developed malignant lesions in the glandular stomach was also open to doubt on the pathological evidence. The effect of feeding rats on a balanced diet containing cholesterol heated to 270° to 300° C. for half an hour was investigated, but no lesions of the stomach were seen that could not be attributed to other causes (16). Further, an attempt to concentrate any potential carcinogen in the heated cholesterol was made by removing some of the known products of pyrolysis; other groups of rats were fed on the residue with equally negative results (17). At the same time, Waterman's assertion (36, 38) that Δ3,5-cholestadiene is carcinogenic was not substantiated in further feeding experiments (17); the noncarcinogenicity of this substance had been shown already by the epidermal and subcutaneous routes by Strong (33). There remained two possibilities that would still allow foodstuffs containing cholesterol to become carcinogenic as a result of heating. In the first place, cholesterol is usually present in foodstuffs as an ester of one or other of the higher fatty acids; these esters might respond to pyrolysis in a manner different from that shown by the free sterol. Evidence that such a difference in behavior is possible lies in the work of Bergström and Wintersteiner (3), who found that cholesterol and its esters, in colloidal suspensions, are oxidized by atmospheric oxygen at considerably different rates. Secondly, although 280° C. is the usual maximum temperature obtainable in a "regulated" gas oven, not all ovens are regulated and, moreover, in grilling and similar procedures the outer layer of the meat, for example, is undoubtedly subjected to a much higher temperature than is usual for other forms of cooking; a temperature above that employed in the earlier experiments (16) might well lead to different results.

* Because of the difficulties of international communication the author has not read proof of this article.

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(A) Experiments with Cholesterol Esters

Esters of cholesterol with two commonly occurring fatty acids, stearic and palmitic, were selected. Both fatty acids were obtainable in a pure state and were converted to the corresponding acid chloride by interaction with thionyl chloride on the water bath, followed by distillation at about 15 mm. The esters were prepared by von Christiani's method (4), which allows cholesterol to react with appropriate acid chloride on the water bath; the product was dissolved in chloroform, washed with carbonate, dried and precipitated with petroleum ether (b.p., 60-80° C.). In this way esters were obtained that were free from pyrolytic products to begin with. The palmitic ester had a melting point of 78° C., while the stearate had a melting point of 83° C.

In either case, the ester was heated in an open beaker on an electric hot plate to approximately 300° C. for half an hour. The molten mass was allowed to cool considerably and was then poured into petroleum ether (b.p., 60-80° C.). This solution was chilled; unchanged ester was filtered off and subjected to a further heating. This was continued until no unchanged ester could be recovered. The rate of pyrolytic change was somewhat, but not very much, greater than in the case of the free sterol heated in the same way. Thus, of 97 gm. cholesterol stearate heated as described, 70 gm. unchanged ester was recovered after one heating, *i.e.,* about 25 per cent had been altered, as compared with 16 per cent in the case of the free cholesterol (16).

The combined petroleum ether solutions were, in either case, concentrated on a water bath and finally in a vacuum desiccator. The hard gum remaining was weighed and dissolved in enough chloroform to yield a 25 per cent solution. Pieces of compressed rat-cake (35) were impregnated with 25 mgm. of heat products by allowing 0.1 ml. of the chloroform to evaporate on each piece.

Twenty-four Wistar rats, about 3 months old, received a basal diet of rat-cake and water *ad libitum,* with green stuff and carrot once weekly. Twelve rats received daily 25 mgm. (as described, on rat-cake) of the heat products from cholesteryl palmitate; the other 12 rats received cholesteryl stearate heat products. After 15 months, the feeding of heat products was stopped and the remaining animals allowed to live as long as they could. It was reasonable to suppose that 15 months' exposure to a carcinogen would be enough to elicit a detectable reaction providing the total life was long enough.

Results with esters.—Seven of 12 rats receiving cholesteryl stearate heat products survived for 450 days (*i.e.,* the period of administration of the heat products); only 1 of these had lungs free from congestion or bronchiectasis at the time of death. Two had tapeworm cysts in the liver, and one had an abscess of the left ovary. The spleen was usually examined microscopically and was found to show considerable reduction of lymphoid tissue and of germinal centers, while the reticuloendothelial cells were greatly increased. In no rat stomach was any abnormality of the *pars squamosa* observed; the only lesions seen in the *pars glandularis* were the hemorrhagic punctate erosions, often extending throughout the depth of the epithelium but not affecting the submucous layers, which seem to be a starvation phenomenon (23) and were usually present in these experiments whenever the lungs had extensive lesions. Two rats survived for 590 days.

Of the rats receiving cholesteryl palmitate heat products, 9 of 12 survived 450 days. Emphysema or bronchiectasis was present in all rats. Among 6 rats, 2 had liver tapeworm cysts, 1 a liver infarct, 2 some localized fatty degeneration of the liver, and 2 a few small focal necroses of the liver. Most spleens showed changes similar to those recorded above. Several rats had hemorrhagic erosions in the glandular part of the stomach, but 2 showed a slight hyperkeratosis (at 372 and 676 days) and 1 of these a possible early papillomatosis (at 372 days) of the forestomach. Four rats survived 640 days or more.

Thus it will be seen that no significant lesions of either portion of the stomach, nor any tumor in any part of the body, have been caused by feeding the products of heating cholesteryl stearate or cholesteryl palmitate to 300° C. for half an hour during a period of 15 months, even though some rats survived nearly 2 years.

(B) Experiments with Cholesterol Heated to 430° C.

The top heat of the electric plate available was sufficient to raise cholesterol melted in a beaker to 430° C., and this temperature was therefore used for investigating the effect of heating cholesterol to a temperature rather above the normal cooking maximum. Cholesterol, obtained from Glaxo Laboratories, Ltd., was purified by conversion to the acetate, which was recrystallized from ethanol and saponified with potassium hydroxide in methanol (to avoid contamination with resinous by-products); the cholesterol was then recrystallized from petroleum ether (b.p., 60-80° C.), until the mother liquor was nonfluorescent in the ultraviolet beam. Batches of this purified cholesterol were heated for half an hour to 430° C. It was found that nothing crystallized out when the molten mass was poured into petroleum ether; hence it was concluded that, unlike heating to 300° C. for half an hour, heating to 430° C. for this period altered most

of, if not all, the cholesterol. The viscous oil formed by heating was dissolved in chloroform to yield a 25 per cent solution; this was dropped onto rat-cake in the usual way, so that each piece of the rat-cake held 25 mgm. of the heat products.

Twelve Wistar albino rats were kept on the basal diet used in the previous experiments, and also received 1 piece each of impregnated cake, *i.e.,* 25 mgm. heat products daily. Feeding with the test material in this experiment was continued throughout the life of the animals.

Results with cholesterol heated to 430° C.—Eleven of 12 rats were examined post mortem. Pneumonia or bronchiectasis was present in 9, and an increase of reticuloendothelial elements accompanied by a decided loss of lymphoid tissue was seen in the spleen of several rats. Two rats had encysted tapeworms but no other lesion in the liver. Four rats, dying at 392, 461, 509, and 529 days respectively, had no abnormality of either part of the stomach; 2 others, dying at 460 and 532 days respectively, had normal forestomachs and only hemorrhagic erosions of the type previously recorded in the glandular zone. Of the other 5 rats, 2, dying at 380 and 467 days respectively, had normal glandular stomachs but the forestomach showed a small degree of hyperkeratosis; a third, dying at 411 days, had hyperkeratosis of the forestomach and deep erosions (not affecting the submucous layers) of the glandular mucosa. Hyperkeratosis is probably common in the forestomachs of old rats, but these rats could not be considered old. Two rats died at 296 and 318 days respectively after the experiment was begun, *i.e.,* before any of the others; both of these had macroscopically dry-looking stomachs with very prominent blood vessels. Microscopically, there were seen in these two stomachs fibrogranulomatous lesions of the muscle wall and serous coat, calcified in places; the lesion was thought to be of parasitic origin and not to be directly related to the experiment.

No tumor of any sort was seen in any animal of this group.

DISCUSSION

It will be seen from the results recorded above that no stomach lesion that could not be attributed to other causes, nor any tumor of any other part of the body, was induced in rats fed with either cholesterol esters that had been heated to 300° C. for half an hour, or cholesterol itself that had been heated to 430° C. for half an hour. Roffo reported, with numerous illustrations, malignant lesions of the glandular stomach and also sarcomas of the mesentery and liver, in rats fed cholesterol that had been heated to 350° C. for half an hour. It is not possible to discover from Roffo's papers at just what level he fed his cholesterol heat products, and it is possible that this level greatly exceeded that employed in the present series of experiments. In the case of rats fed with cholesterol heated to 430° C., the fact that none of them survived 18 months leaves open the question whether tumors might or might not have developed late in the life of the animals; on the other hand, the temperature employed, 430° C., was well above that used by Roffo, 350° C., and any effects produced by the heat products should have appeared rather earlier in these experiments than in Roffo's, where 16 months seems to have been the minimal latent period.

Roffo's statements on mesenteric sarcomas induced in rats by the ingestion of heated fats seem to be substantiated by the work of Peacock and Beck (24), who found multiple mesenteric tumors in 4 of 71 rats that had been fed with lard heated to 220° C. or 350° C. for at least 300 days. But there is no evidence from the experiments recorded here or previously (16, 17) that cholesterol or its esters supplied the sarcogen. The objections raised by Klein and Palmer (18) to Roffo's assertions regarding his rat stomach lesions, and the failure of Beck and Peacock (2) to obtain any stomach tumors in rats fed with heated fats up to 436 days, coupled with the results of this series of experiments, lend no support to the theory that heated fats, or heated cholesterol or its esters, added to the diet of rats will induce malignant tumors of the glandular stomach.

The question whether heating cholesterol to 300° C. or more produces a carcinogenic substance is not entirely settled. As Kennaway and Sampson showed (15), cholesterol distilled over pumice at 800° C. in an atmosphere of hydrogen yields a carcinogenic tar, but the pyrolytic conversion to a carcinogen may begin at a much lower temperature. Steiner, Steele, and Koch (31) investigated the "possible carcinogenicity of . . . heated cholesterol"; they heated purified cholesterol for 2 hours at 200° C., and also at 300° C., and injected totals of 200 mgm. per mouse subcutaneously in mice known not to develop sarcoma spontaneously. Eight mice survived for a year or more, but no tumors were found, although sesame oil that had been heated to 350° C. did induce sarcomas in 3 of 9 mice from the same strain that survived 1 year; Beck (1) also obtained sarcomas by the subcutaneous injection of heated cottonseed oil into mice.

The possible carcinogenic action of heated cholesterol has also been investigated in this Department. Solutions in olive oil or arachis oil of the various pyrolytic products described in this and preceding papers (16, 17) were injected subcutaneously into stock mice by Dr. S. Beck, who tested some of the products by painting also on similar mice. The results will be published separately,[1] but it can be stated here that

[1] See following paper.

while no malignant tumors have been obtained with cholesterol heated to 430° C., either by painting or by injection, the injection experiments with some of the products obtained at 300° C. have yielded a few fibrosarcomas. The total number of animals used was too small to evaluate the significance of these tumors, but the spontaneous development of sarcoma in these mice has not been observed here. There is therefore some biological evidence that cholesterol or its esters, when heated to 300° C. for half an hour, may yield products containing a weak carcinogen.

Further Chemical Investigations

It was reported in Part I (16) of this series that cholesterol heated to 300° C. acquires a decided blue-violet fluorescence in the ultraviolet beam, and that cholesterol heated to 440° C. is still richer in this fluorescent material. Hexane solutions were purified by passage down towers of activated alumina and filtrates were obtained having a three-banded fluorescence spectrum, reminiscent of that of 20-methylcholanthrene; absorption spectra, however, showed maxima at 2,330, 2,440, 2,560, and 2,615 A, but no trace of a band in the region of 3,000 A, where all known carcinogenic polycyclic hydrocarbons seem to show strong absorption (14, 20, 21). An attempt was made to clarify this situation.

100 gm. purified cholesterol was heated to 430° to 440° C. in a 250 ml. conical pyrex flask fitted with a cork stopper carrying a thermometer, an outlet tube, and an inlet tube that reached down nearly to the level of the molten cholesterol. A slow stream of air was sucked through the apparatus and the pyrolysis products were led through two boiling-tubes, in series, cooled in ice water. Water and mobile, colorless distillates appeared in the early stages; a second nonfluorescent distillate was also removed. Three further arbitrary fractions were collected during an hour or so; all separated into 2 layers in the boiling-tubes, the lower layers being greenish in daylight but blue-violet fluorescent in the ultraviolet beam. The residue in the flask weighed 6 gm.; 4.5 gm. was insoluble in chloroform and was mainly carbon.

The fluorescent layers were combined and dissolved in ether. The ethereal solution was washed with 2 *N* NaOH and then with 2 *N* HCl; the latter extracted no fluorescent material, but the alkaline washings were colored and not entirely nonfluorescent; they were therefore acidified, extracted with ether, and the extracts combined with the main ethereal solution, which was then transferred into petroleum ether (b.p., 60-80° C.). This solution was poured through activated alumina whereby considerable colored material was removed; further purification was achieved by chromatograms carried out with *cyclo*hexane solutions, but the brightly blue-violet fluorescent solutions were still yellow in daylight.

The material was then distilled at 15 mm. and three fractions of increasing viscosity were obtained: (a) up to 170° C., (b) 170° to 220° C., (c) up to 270° C. All were fluorescent, (a) being nearly colorless. Further chromatograms were carried out in the hexane solution but the filtrates, though fluorescent, were still yellow. Attempts to prepare a picrate at this stage failed.

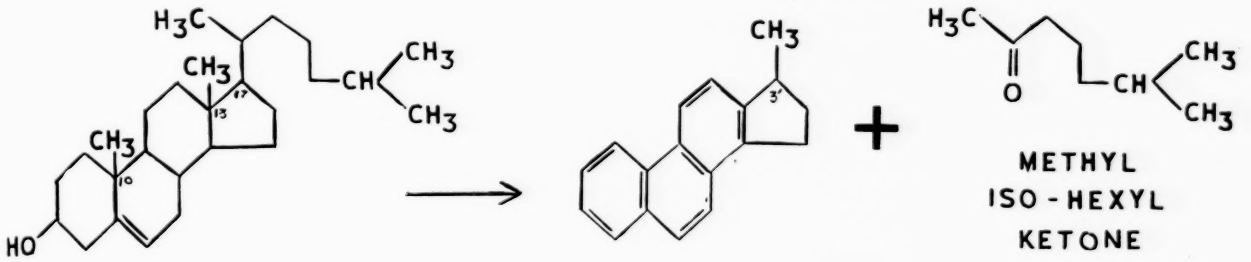
The fractions were combined and passed repeatedly through alumina, in hexane solution. About 48 gm. of a pale yellow, intensely blue-fluorescent material was now in hand. This oil was distilled at 0.1 mm. and three fractions obtained; (a) up to 140° C., colorless, blue-fluorescent; (b) 140° to 200° C., pale yellow, blue-fluorescent; (c) 200° to 240° C., yellow, blue-fluorescent. A fluorescence spectrogram was made, using a K.B.B. mercury vapor lamp with a Wood's glass screen, of all fractions in *n*-hexane and of 20-methylcholanthrene; fraction (c) resembled the hydrocarbon most, but in all cases the bands were shifted towards the mercury line at 3,650 A. No picrate could be obtained from fraction (c), nor any acid insoluble material by oxidation with dichromate in acetic acid solution. Chromatograms were carried out in ether and *n*-hexane, but a completely colorless filtrate was never obtained.

At this stage, Dr. E. R. Holiday, of the Department of Biochemistry, Oxford, kindly undertook to examine the fluorescent filtrate by his moving plate method (13), which was expected to reveal a polycyclic hydrocarbon even in the presence of contaminants having general absorption. Dr. Holiday reported that the absorption spectrum was that "of an almost pure compound very closely related to 1:2-*cyclo*pentenophenanthrene." The process of pyrolysis had been accompanied throughout by a strong smell of orange oil. This odor has been observed by various workers in the course of oxidations of cholesterol; Dorée and Gardner (9) noticed it when oxidizing cholesterol with hydrogen peroxide. Diels (7) suggested that it was methyl*iso*hexylketone, and Windaus and Resau (39) isolated this ketone in the form of its semicarbazone in 1913. It is formed by the breaking off of the entire side chain from the cholesterol molecule (Fig. 1). This leaves the *cyclo*pentenophenanthrene nucleus in the hydrogenated condition, and with the angular methyl groups at carbon atoms 10 and 13. In 1927 Diels, Gädke, and Körding (8) isolated a hydrocarbon from the hydrogenation products of cholesterol, which Hillemann (11) ultimately showed to be 3′-methyl*cyclo*pentenophenanthrene. It is assumed (30) that when the side chain breaks away from the cholesterol molecule the methyl group at C_{13} migrates

to C_{17}, while the other methyl group is lost together with the hydroxyl group at C_3 and several atoms of hydrogen, yielding the completely unsaturated hydrocarbon, $C_{18}H_{16}$.

The formation of the methyl*iso*hexylketone in the distillate from cholesterol heated to 430° C. would therefore open the way for the formation of the Diels hydrocarbon, $C_{18}H_{16}$. Dr. Holiday concurs that the absorption spectrum of the fluorescent filtrate corresponds exactly with that of 3′-methyl*cyclo*pentenophenanthrene published by Rosenheim and King (30). He was unable to find any other substance showing specific absorption. From this it must be concluded that the bands previously reported at 2,330, 2,440, 2,560, and 2,615 A. were due to benzene present in the *n*-hexane used for chromatograms, which had been carried over into the solution used for the absorption spectra. Nevertheless, the fluorescence remains unaccounted for, since Hillemann (12) states that pure 3′-*cyclo*pentenophenanthrene is not fluorescent, a fact that Professor G. A. R. Kon has confirmed (private communication). A sample of the Diels hydrocarbon, prepared from cholesterol by Professor J. W. Cook and kindly given to the author by Dr. O. Rosenheim, was found to be blue-fluorescent. A fluorescence spectrum of this sample, in *n*-hexane, showed three wide bands centering on 3,850, 4,100, and 4,300 A; there was also a faint band at 3,650, just beside the mercury line. The three long-wave bands were practically identical with those given by the fluorescent filtrate in *n*-hexane, but not with those given by 20-methylcholanthrene or 3,4-benzpyrene. Hence these results lend no support to Roffo's assertions regarding the formation of 3,4-benzpyrene or other known carcinogenic hydrocarbons by the action of heat on cholesterol.

The pyrolysis of cholesterol, therefore, appears to lead to the formation of dicholesteryl ether and Δ4-cholestenone (16), pseudo-cholestene (10), methyl*iso*hexyl ketone, and 3′-methyl*cyclo*pentenophenanthrene. Of these substances, the latter is the only one to have been suspected of carcinogenic power. Craciun, Zugrăvescu, Ursa, and Manulescu-Motoc, according to an abstract in the Chemisches Zentralblatt (6), said that, of the hydrocarbons prepared by Diels, only the 3′-methyl*cyclo*pentenophenanthrene is carcinogenic. This statement requires confirmation, but if it is true the repeated ingestion over many years of even traces of this substance in fried food, for example, may have pathological significance.



CHOLESTEROL DIELS' HYDROCARBON. $C_{18}H_{16}$

FIG. 1

SUMMARY

1. Rats fed an adequate basal diet developed no significant lesions of the stomach nor tumors of any part of the body when fed with the products of heating either cholesteryl stearate or palmitate to 300° C. for half an hour at 25 mgm. per day level for 15 months, and allowed to live out their natural span.

2. Similar rats, fed with the same basal diet plus 25 mgm. daily of the products of heating cholesterol for half an hour to 430° C., showed only a slight degree of hyperkeratosis of the forestomach and no tumors of the glandular zone, nor any tumors in any other part of the body.

3. The pyrolysis of cholesterol at 430° C. has been shown to break the molecule at C_{17}, yielding methyl*iso*hexylketone and the Diels hydrocarbon, $C_{18}H_{16}$, which may be carcinogenic. The fluorescence of the pyrolysis product is not yet accounted for.

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Tumors Induced with Heated Cholesterol*

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Roffo, in a series of communications (10-13), asserted that rats fed for long periods with fats or cholesterol that had been heated to 350° C. for half an hour developed a variety of tumors, including adenocarcinoma of the stomach and sarcomas of the liver and peritoneum. These papers were copiously illustrated with photomicrographs and drawings, from which it appears that many sarcomas occurred in the experiments; but the evidence for adenocarcinoma is less convincing and has been criticized severely by Klein and Palmer (8). An attempt to repeat Roffo's work, with lard and lard substitutes, was made by Beck and Peacock (3), who described a condition of gastropapillomatosis of the forestomach accompanied by other signs of avitaminosis A; it was shown that the diet containing heated fat induced avitaminosis A, and that the gastric lesions could be prevented or cured by adding carrot to the diet. No lesions were seen in rats receiving the same basal diet plus unheated fat at the same level. Malignant tumors of the stomach, or of the alimentary canal, were not seen in these experiments.

Subsequently, also from this laboratory, the results of feeding rats with supplements of heated cholesterol have been reported. Cholesterol was heated for half an hour to 300° C. and fed at a 20 mgm. per rat per day level for up to 2 years (5). Two definite pyrolytic products were separated from cholesterol that had been heated to 300° C. for half an hour, and the residue was also fed to rats at a 20 mgm. per rat per day level up to 2 years (6). Another possible product of pyrolysis, Δ3,5-cholestadiene, which Waterman (19) and Veldstra (18) have postulated as the cause of the carcinogenic action of heated fats, was tested by feeding rats with it at a 25 mgm. per rat per day level for 2 years (6); by painting and injection Strong (17) found this diene noncarcinogenic for mice. Two other possible mechanisms whereby heating might render cholesterol carcinogenic have been tested, and the results are now available (7); esters of cholesterol, heated for half an hour to 300° C., were fed at a 25 mgm. per rat per day level for 15 months and the rats then allowed to live as long as possible; other rats were fed 25 mgm. per rat per day of the product of heating cholesterol for half an hour to 430° C. up to 18 months. In none of these experiments did any rat develop any malignant tumor at any site, nor were lesions of the stomach observed that could not be attributed to causes other than the experimental procedure.

It is a fact, however, that some substances are carcinogenic by one route and not by another; some induce epitheliomas more readily than sarcomas, while others show the reverse effect (5). Kennaway and Sampson (4) had shown that cholesterol, distilled over pumice at 800° C. in an atmosphere of hydrogen, yielded a carcinogenic tar, as shown by painting experiments with mice, and it seemed worth while to test the various products of heated cholesterol prepared in this laboratory for carcinogenic activity by the usual cutaneous and subcutaneous routes. Solutions of the various products that were being fed were therefore made in oil for subcutaneous injection; a solution in acetone of cholesterol heated to 430° C. was used for painting, while cholesterol heated to 300° C. was painted on in oily solution. The details of the various products used and the solvents and strengths employed are set out in Table I.

Biological Experiments and Results

Stock mice were used in all experiments. All injections were given subcutaneously into the right flank, on one occasion only. Painted solutions were applied twice weekly between the shoulder blades, after the hair had been clipped away.

Solution 1a.—Five-tenths milliliter of solution 1a (Table I) was injected into each of 18 mice. A very small tumor was noticed by palpation in a mouse of this group at the site of injection on the 386th day. The mouse was sacrificed shortly afterwards, when it was found that the tumor originated in the wall of a cyst still containing some of the injected material. Histologically, the tumor was a spindle-cell sarcoma

* Because of the difficulties of international communication the authors have not read proof of this article.

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(Fig. 3). Two lung adenomas were found in this group of mice.

Solution 1b.—Twelve mice were painted with solution 1b (Table I) throughout their lifetime. There was only very little depilation, and no papilloma nor malignant tumor was observed in the painted area. Multiple mesenteric tumors were observed in 1 mouse of this group.

Solution 2.—Five-tenths milliliter of solution 2 (Table I) was injected into each of 12 mice. One spindle-cell sarcoma (Fig. 4), penetrating the muscle and peritoneum and covering the site of injection, was found after 345 days. No spontaneous tumors appeared in this group.

Solution 3.—Eighteen mice were each injected with 0.5 ml. of solution 3 (Table I). No tumor at any site was observed in this group.

Solution 4.—Twenty-four mice were each injected

TABLE I

Substance	Solution for injection: Solvent	Solution for injection: Strength	Solution for painting: Solvent	Solution for painting: Strength	Local tumor incidence
1a Cholesterol heated to 270-300° C.*	Olive oil or cottonseed oil	20%	—	—	1/18 §
1b "	—	—	Cottonseed oil	20%	0/12
2 Residue from substance I. †	Olive oil	20%	—	—	1/12 §
3 Cholesteryl stearate heated to 300° C. ‡	Arachis oil	20%	—	—	0/18
4 Cholesteryl palmitate heated to 300° C. ‡	Arachis oil	25%	—	—	0/24
5a Cholesterol heated to 430° C. ‡	Arachis oil	20%	—	—	0/18
5b "	—	—	Acetone	17%	3/18 ‖

* Preparation described in (5).
† " " " (6).
‡ " " " (7).
§ Spindle-cell sarcoma.
‖ Papillomas.

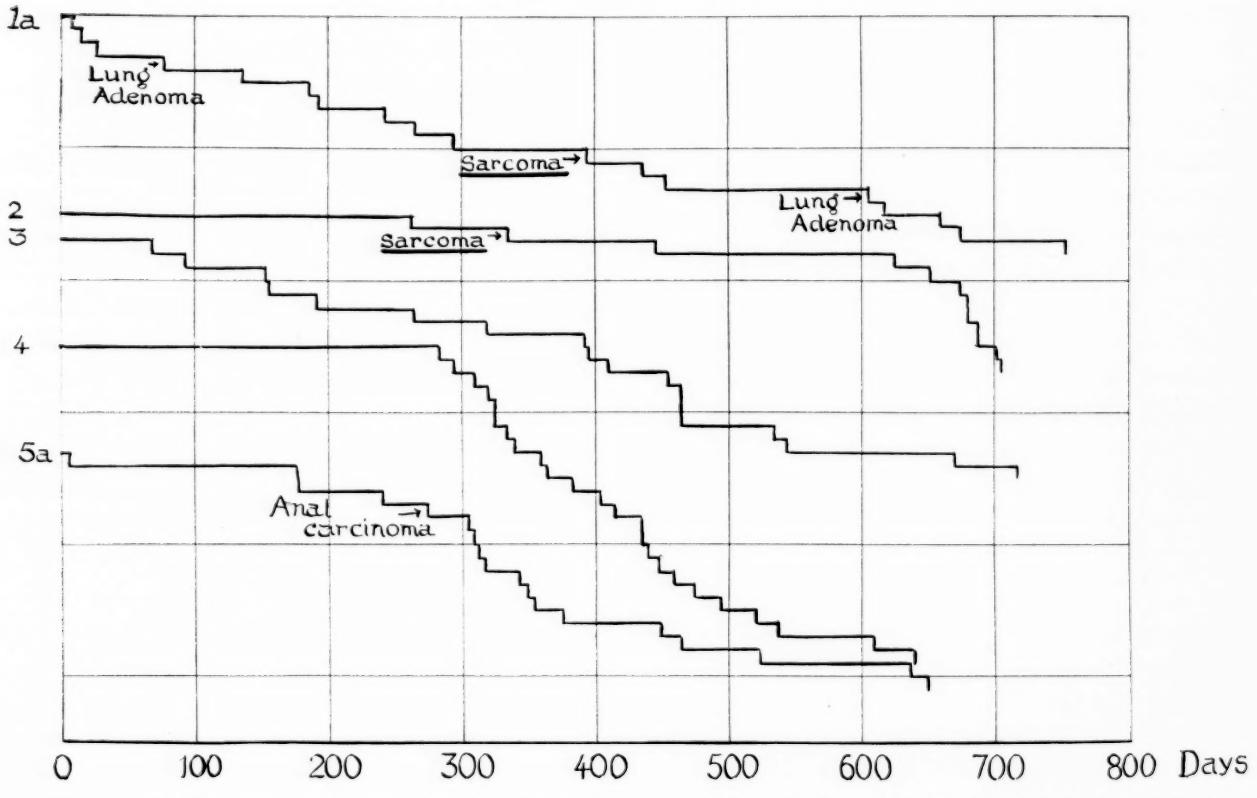

FIG. 1.—Mortality curves and tumor incidence for injection experiments; local tumors related to injections underlined.

with 0.5 ml. of solution 4 (Table I). No tumor was observed at the site of injection in any mouse, but a malignant growth of the cheek occurred in 1 mouse of this group.

Solution 5a.—Eighteen mice were each injected with 0.5 ml. of solution 5a (Table I). No tumor occurred at the site of injection in any mouse, but a squamous carcinoma of the anus was observed in 1 mouse of this group.

Solution 5b.—Eighteen mice were painted with solution 5b (Table I) during their lifetime. No carcinoma occurred, but 3 papillomas were observed in this group, the earliest after 460 days. Two of the papillomas regressed but 1 persisted until the animal died at 584 days [1] (Fig. 5). The papillomas did not invade the skin, and were of a different type from that associated with the application of the well-known carcinogenic hydrocarbons, being unbranched, simple papillomas.

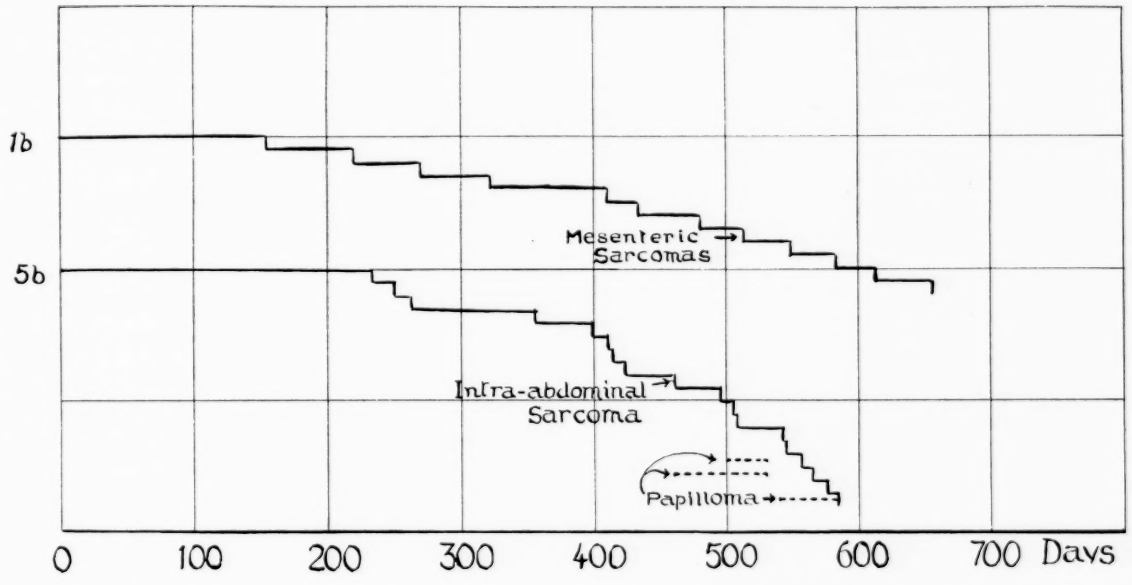

FIG. 2.—Mortality curves and tumor incidence for painting experiments; local tumors related to painting underlined.

DISCUSSION

The high incidence of cancer of the alimentary canal, and particularly in the stomach, of man as contrasted with other species, has led many investigators to search for extrinsic causes peculiar to human communities. Food is the most obvious vehicle for extrinsic carcinogenic factors. Heated fats and cholesterol have been incriminated by Roffo and others, but his evidence for the experimental induction of adenocarcinoma of the stomach has not been independently confirmed. If the natural evolution of adenocarcinoma of the stomach in rodents resembles the disease in man, one would expect to find invasion of local lymph nodes by the tumor and metastases in the liver, but neither of these has been recorded. It must be recognized, however, that until quite recently no experimentally induced adenocarcinoma of the rodents' stomach was available for comparison. But since Stewart (15), using Strain A mice, and Stewart and Lorenz (16), using mice of the C3H, I, and C57 brown strains, have successfully induced this type of neoplasia by intramural injection of methylcholanthrene in the stomachs of mice, study of the disease in animals has become possible. So far as we are aware, no one has induced adenocarcinoma of the stomach in any animal by feeding even one of the potent synthetic carcinogenic hydrocarbons, though adenocarcinoma of the small intestine is reported by Lorenz and Stewart (9) in Strain A mice fed with methylcholanthrene in aqueous-oil emulsion. It is not to be supposed, however, that human patients who develop cancer of the stomach come in contact with comparable concentrations of such potent carcinogens, if, indeed, they ingest them at all. One must be prepared, therefore, to consider seriously as a possible etiological factor in the human disease any substance that can be shown to be carcinogenic and that can be extracted from a common article of diet. It is found, experimentally, that feebly carcinogenic hydrocarbons induce tumors in animals after a longer latent period than do the more potent substances, such as methylcholanthrene; but even where the latter are concerned, dilution can prolong the latent period and reduce the proportion of positive results.

[1] The stock of mice were suffering from an acarine infestation at this time, and a burrow containing a parasite can be seen in Fig. 5 at the edge of the papilloma. Similar burrows in other mice were not associated with any type of new growth. The association in this case is thought to be fortuitous.

The known facts about gastric cancer in man are quite compatible with the conception of a weak carcinogen acting over a latent period of 30 years or more. Positive evidence that heated fats injected into the connective tissue can induce sarcoma in mice is available from several independent sources: Beck (2), Steiner (14), and others. The experiments of Stewart and Lorenz (16) show that local circumstances may allow a substance that can induce sarcoma or epithelioma, depending on the mode of exhibition, to induce adenocarcinoma provided that it be retained in proximity to the secreting epithelium. No substance, therefore, that can be shown to induce malignant neoplasia at any site can be dismissed as noncarcinogenic for other sites. Such a simple physical barrier as immiscibility of a fat with watery secretion might prevent indefinitely the local action of a carcinogen. It seems possible that fat-soluble carcinogens might be more liable to affect areas of atrophic glandular epithelium than similar normal areas, owing to the flow from the latter of watery mucous secretions. An attempt was made in this laboratory by one of us (S. B.) to induce atrophic gastritis in rats by restriction of the diet, with a view to subjecting them to carcinogens administered in the drinking water. Atrophic lesions were obtained, but the rats did not live long enough for the test to be carried out. In our experiments, two sarcomas were observed in mice following the subcutaneous injection of a solution in arachis oil of cholesterol that had been heated to about 300° C., and papillomas occurred in 3 mice painted with similar material. The incidence of these tumors points to a low-grade carcinogenicity of heated cholesterol or its esters, but this is of some practical interest for the conditions of the experiment

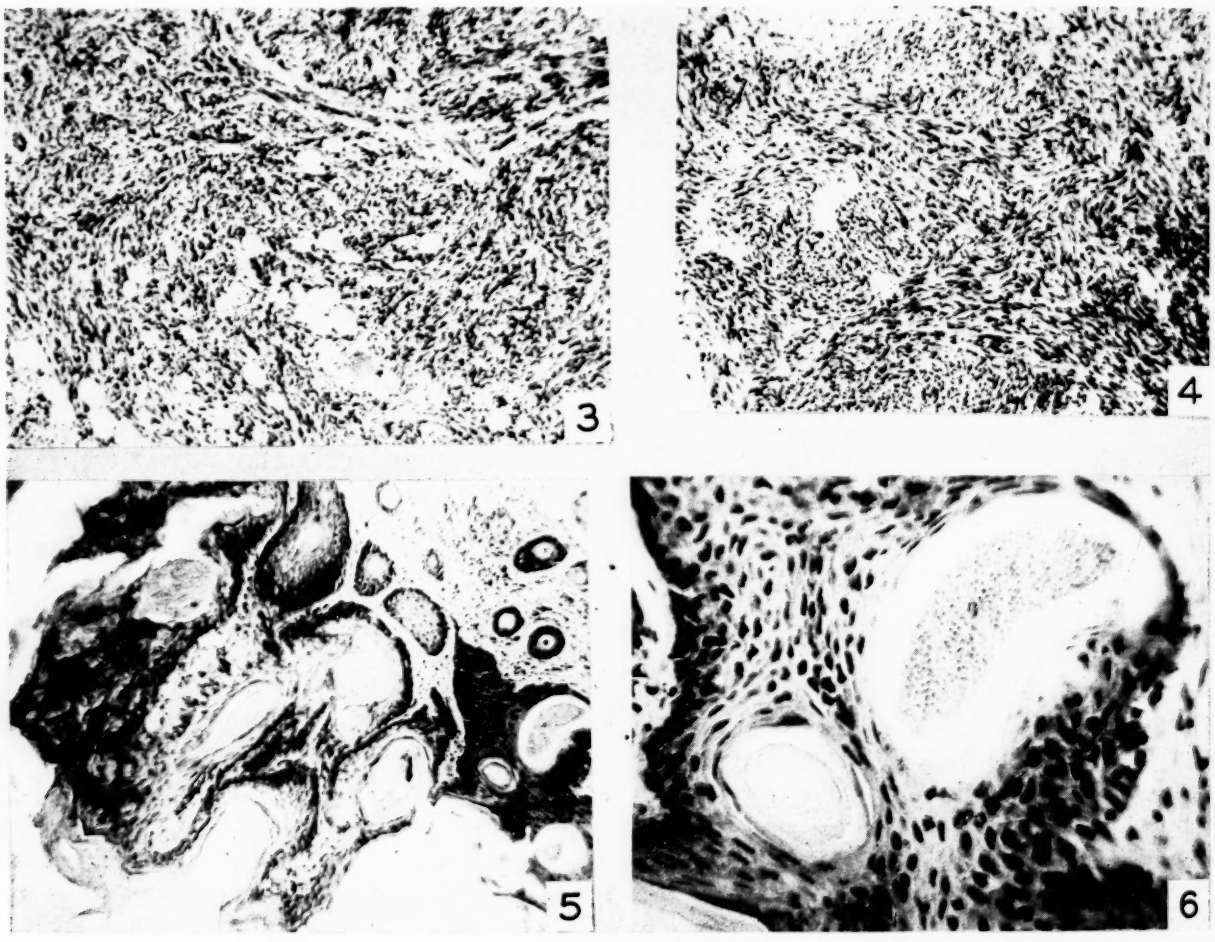

FIG. 3.—Spindle-cell sarcoma at site of injection, after 386 days, in a mouse injected with cholesterol heated to 300° C. (solution 1a).

FIG. 4.—Spindle-cell sarcoma at site of injection, after 345 days, in a mouse injected with a residue from cholesterol heated to 300° C. (solution 2).

FIG. 5.—Simple papilloma at site of painting, after 460 days, in a mouse painted with cholesterol heated to 430° C. (solution 5b); a burrow of acarine origin can be seen at the right-hand edge.

FIG. 6.—Enlargement of part of Fig. 5, showing the acarus burrow.

as regards the heating of the cholesterol were such as might occur in cooking food. In this connection it should be noted that standards of carcinogenicity referred to such substances as 3,4-benzpyrene or methylcholanthrene tend to minimize the importance of very much weaker carcinogens, such as we are dealing with at present, which may, in consequence, be regarded as of little importance. This is probably the wrong way to look at the problem, as we have no evidence that human beings come in contact with methylcholanthrene outside special laboratories, and, even in the case of 3,4-benzpyrene, contact with the substance is probably limited to certain industries. The establishment of the carcinogenicity, however weak, of any substance that forms a regular article of human diet cannot be regarded as insignificant. It may well prove, as in the case of the relationship of benzpyrene to tar, that the heated cholesterol fractions with which we are dealing contain only a minute concentration of a carcinogen that might be potent in the pure state.

SUMMARY

1. Stock mice were injected with oily solutions of the following substances: (a) cholesterol heated to 270° to 300° C. for half an hour; (b) the residue left from (a) after dicholesteryl ether and Δ4-cholestenone had been removed; (c) cholesteryl stearate heated to 300° C. for half an hour; (d) cholesteryl palmitate, similarly treated; (e) cholesterol heated to 430° C. for half an hour.

2. Similar mice were painted with (a) cholesterol heated to 270° to 300° C. for half an hour, dissolved in cottonseed oil; (b) cholesterol heated to 430° C. for half an hour, dissolved in acetone.

3. Cholesterol heated to 270° to 300° C. produced a spindle-cell sarcoma originating in the wall of the injection cyst, after 386 days. The residue left from the cholesterol produced a spindle-cell sarcoma after 345 days.

4. Painting with cholesterol heated to 430° C. produced 2 regressing papillomas and 1 persistent papilloma, the earliest papilloma occurring after 460 days.

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The Response of the Central Nervous System of the Rat to Methylcholanthrene

I. The Induction of Tumors Derived from Nervous Tissue*

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INTRODUCTION

Seligman and Shear (15) in 1939 reported the induction of 11 glial tumors in C3H mice with methylcholanthrene, though all previous attempts to produce neoplasia of the cells of the nervous system in mice (7), guinea pigs (2), rats (10, 15), and rabbits (3) with carcinogenic agents had been unsuccessful. Following the work of Seligman and Shear, Zimmerman and Arnold (20), and Peers (12) reported the induction of glial tumors in mice with methylcholanthrene. The tumors elicited in most instances were remarkably similar to some types of human glioma, and all the varieties of glial tumors observed in man have now been induced. More recently glial tumors have been elicited in mice with benzpyrene (21) and with dibenzanthracene (1).

Attempts to induce neoplasia in the nervous system of the rat with carcinogenic agents have not been attended with the same success as in mice. In 1936 Oberling, Guérin, and Guérin (10) applied benzpyrene in a crystalline form to the brains of 10 adult rats. After a 10 month period adenomas of the pituitary gland, believed by the authors to have been caused by the carcinogen, were noted in 3 of the animals. Three years later Oberling, Sannié, and Guérin (11) reported a more extensive study, in which an oily solution of benzpyrene was injected into the brains of chickens, guinea pigs, rabbits, and rats. No pituitary adenomas were observed in any of the animals, and the previous statement, that benzpyrene produced adenomas of the pituitary, was retracted. Several animals survived more than a year, but no glial tumors were found, although 3 of the rats developed fibrosarcomas.

In a personal communication to Seligman and Shear, Zylberszac (22) told of experiments with 35 rats in which crystalline benzpyrene was placed beneath the dura over the frontal lobes of the brain. No tumors were noted after a period of 14 months. In a similar communication to Seligman and Shear, Scherer (14) reported injecting 0.1 cc. of a 1 per cent solution of benzpyrene in lard into the frontal lobes of 70 white rats. Fifty of the animals were given more than one injection. The experiment was terminated after 300 days, and 27 of the rats lived longer than 200 days. No true neoplasms were noted, but in every rat a granulomatous foreign body reaction developed around the injected material.

In 1938 Weil (18) reported experiments in which he injected a solution of lard and dibenzanthracene into the brain of white rats. Following a period of 7½ months there developed in one rat a "squamous epithelial carcinoma" surrounded by active glial proliferation. Apparently Weil regarded this lesion as true neoplasia, and not merely hyperplasia, because in discussing a paper by Sweet and Bailey in 1941 (17) he emphatically stated that it was a glial type of tumor. He took issue with Peers (12), who, in a communication referring to Weil's work, had questioned "a direct causal relation between this mass and the implanted chemical." In the same discussion Weil mentioned that subsequently he had "observed small gliomas in the rat brain after implantation of methylcholanthrene crystals and a large glioma of the spinal cord in combination with an intradural sarcoma." Details of these experiments were not given.

Sweet and Bailey reported the implantation of crystals of methylcholanthrene directly into the cerebral hemispheres of 42 white rats of the Wistar strain of varying ages, from 4 weeks to maturity. Five of the rats developed tumors. One of the growths, a glioma with the histologic characteristics of a spongioblastoma multiforme, developed 8 months after implantation of the carcinogen. The other 4 tumors were fibroblastic in nature.

In discussing the paper of Sweet and Bailey, Greenwood mentioned experiments with 19 rats that received intracerebral injections of methylcholanthrene. Six

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tumors developed, one of which was a glial neoplasm that was classified as a spongioblastoma multiforme. Four of the remaining growths were fibroblastic, and 1 was classified as an adenoma.

It would appear from the foregoing that the cells of the central nervous system of the rat are less responsive to carcinogenic stimulation than those of the mouse. In view of the success that attended the experimental production of glial tumors with carcinogenic agents in mice when a high concentration of carcinogen was employed directly in contact with the nervous tissue for long periods of time (1, 12, 15, 20, 21), the essentially negative results obtained in the rat were surprising. It should be noted, however, that identical procedures and methods were not generally employed in the experiments with rats. In some instances the carcinogen was applied only to the surface of the brain (22) or it was used in an extremely dilute form and mixed with an oily vehicle that produced a foreign body type of reaction (14).

In the experiments reported in this communication with rats, which took into account those factors that proved successful in the production of glial tumors in the mouse, a variety of neoplasms derived from nervous tissue were produced.

Not wishing to rely wholly on an improved technic, the author decided to add still another possible augmenting factor. The work of Kinosita (9), demonstrating a dietary factor in the production of carcinoma of the liver induced in rats with *p*-dimethylaminoazobenzene, and the later work of Kensler, Sugiura, Young, Halter, and Rhoads (8), who showed that riboflavin and casein can protect the liver against the induction of carcinoma by this compound, suggested the employment of a similar dietary factor to influence the development of tumors from nervous tissue in the rat with methylcholanthrene. Accordingly, thiamine and riboflavin were periodically removed from the diet of rats bearing intracerebral pellets of methylcholanthrene. The following communication is a morphologic and histologic study of the tumors produced in these experiments. The effect of the periodic removal of thiamine and riboflavin from the diet will be the subject of another communication (13).

EXPERIMENTAL PROCEDURE

Eighty-five young male and female albino rats of the Rockland strain, about 3 months of age and weighing approximately 120 gm., were employed.

Carcinogen

The methylcholanthrene was obtained from the Eastman Kodak Company. Pellets of 30 per cent concentration were made after the technic used by Peers. Seven parts of chemically pure cholesterol were fused with 3 parts of methylcholanthrene by gentle heating in a sand bath until they were melted and thoroughly mixed. The molten material was then drawn up into a 2 mm. glass tube that had been previously lubricated with mineral oil. After cooling, the solidified mixture was forcibly pressed out of the tube by a small wooden rod. Pellets approximately 3 mm. in length were then cut from the long cylindrical mass, the average weight of each pellet being approximately 25.0 mgm.

Operation

The rats were anesthetized by placing them in a closed glass bowl with cotton saturated with ether. A right paramedian incision was made in the skin of the right parietal region; the periosteum was scraped aside by a sharp scalpel and a hole about 3 mm. in diameter was made through the calvarium with a dental burr. The dura mater was opened by placing a pointed scalpel deep into the hole in the calvarium. With the aid of fine forceps the pellet was pushed through the craniotomy opening and incised dura mater deep into the right cerebral hemisphere. After insertion, the proximal end of the pellet was pushed laterally, so that if it did tend to be forced back it would not be extruded through the wound, since it would be beneath the intact bone and away from the trephined opening in the skull. The skin margins were then approximated by one or two silk sutures and the wound was covered with a thin layer of cotton soaked in collodion. Following the operation, the rats were covered lightly with woolen cloth until they had recovered from the anesthesia. Ten rats died as a result of the operation, either from an overdosage of ether or the trauma to the brain from insertion of the pellet. Eight of the rats that survived the operation died from 1 to 4 weeks later from infection of the brain. The animals that did not develop infection recovered promptly from the operation and remained entirely normal, without discernible neurologic sequelae. It was deemed advisable to kill 15 rats that developed "middle ear disease" during the first 6 weeks after the insertion of the pellet, leaving a total of 52 rats for the experiment.

Necropsy and Histologic Technic

Rats that became moribund were killed by ether inhalation and a complete necropsy was performed. In many instances, however, rats were found dead without having shown any previous symptoms of disease. Following its removal from the skull the brain was fixed in neutral 4 per cent formaldehyde (U.S.P. formaldehyde 1:10). If the brain appeared unusually soft as a result of postmortem change it was deemed expedient to fix it *in situ* after the calvarium had been removed. Following adequate fixation the brain was

sectioned in a coronal plane with a razor blade, care being taken to secure a slice approximately 2 mm. thick and containing the pellet for histologic study. Hematoxylin and eosin and Mallory's phosphotungstic acid hematoxylin stains were employed routinely.

RESULTS

Twenty-one of the 42 rats that survived past the time of appearance of the first tumor (153 days) developed an intracranial tumor. There were 14 tumors derived from nervous tissue, 10 from connective tissue, and both types were produced in 3 of the rats. The incidence and histologic classification are given in Table I.

TABLE I: INCIDENCE OF TUMORS AND THEIR CLASSIFICATION

Total number of animals	42
Animals with tumors	21
Animals without tumors	21
Tumors derived from nervous tissue	14
Spongioblastoma multiforme	6
Astrocytoma	2
Oligodendroglioma	1
Unclassified glioma	3
Tumors composed of nerve cells	2
Tumors derived from connective tissue	10
Extracranial fibrosarcoma	7
Intracranial fibrosarcoma	3
Mixed tumors derived from nervous tissue and connective tissue	3

The experiment was complete after 553 days, since by this time all animals except 1 had either developed an intracranial tumor or died. This rat is still active and well, 700 days after intracerebral implantation of the pellet. There was no significant difference in the average survival time for the animals that developed tumors derived from nervous tissue (299 days) and those that developed fibrosarcomas (282 days). Pertinent data are given in Table II. Brief protocols of the rats that developed tumors derived from nervous tissue are given in the following section, since histologic description and classification are so important in this group of neoplasms. The tumors of mesenchymal origin will be considered collectively, as there was great similarity in all.

TUMORS DERIVED FROM NERVOUS TISSUE
SPONGIOBLASTOMA MULTIFORME

Rat No. 52D.—This animal died 206 days after intracranial implantation of the pellet of methylcholanthrene. The pellet was surrounded by a 1 to 2 mm. zone of gray tissue containing just visible brown-red foci of hemorrhage. Histologic examination disclosed a highly cellular tumor that was not sharply outlined from the surrounding brain. The cells were pleomorphic, some with elongated processes, others stellate with short ones. Mitotic figures were occasionally seen and neoplastic giant cells with multiple chromatic nuclei were noted in several areas. About foci of necrosis the tumor cells were arranged in a palisading fashion with their long axes pointed toward the center of the necrosis (Fig. 2). Small foci of recent hemorrhage were seen in several parts of the section. In the phosphotungstic acid hematoxylin stain there were demonstrated many blue staining intercellular glial fibrils.

Rat No. 73C.—This rat died 303 days after implantation of the pellet. At necropsy there was found a finely granular tumor 1 cm. in diameter and projecting slightly from the surface of the right cerebral hemisphere. Sections of the brain disclosed a light gray tumor containing foci of hemorrhage and replacing nearly the entire hemisphere. Many different types of cells were seen in the tumor, the predominant cell resembling a spongioblast. Others resembling astrocytes or astroblasts, as well as unidentifiable glial types, were observed. Scattered through all parts of the growth were large numbers of multinucleated neoplastic giant cells, some containing small, eosinophilic, round or oval, intranuclear inclusion bodies (Fig. 3). Mitotic figures were frequent in all types of cells, and there was active proliferation of the endothelium of the small blood vessels. Throughout the tumor were many small foci of necrosis such as are usually found with recent hemorrhage. The neoplasm diffusely infiltrated the brain and extended into the lateral ventricle, where it surrounded the choroid plexus. A phosphotungstic acid hematoxylin stain disclosed that all the tumor cells, their processes, and the intercellular fibrils stained a deep blue color.

Rat No. 60D.—The animal died 237 days after implantation of the pellet. Sections through the brain revealed a zone of soft gray-white tumor 2 mm. in width surrounding the pellet. This growth was composed of many different types of cells closely packed together. Spindle-shaped cells resembling spongioblasts; polyhedral cells with short processes, resembling astroblasts; and many small cells with scanty cytoplasm and deeply chromatic nuclei were seen. Mitotic figures and neoplastic giant cells were frequent. Irregular foci of necrosis appeared throughout the growth, and the tumor cells were palisaded around the necrosis. A section stained with phosphotungstic acid hematoxylin revealed many blue staining fibrils between the cells in many parts of the tumor.

Rat No. 21C.—This animal died 321 days after insertion of the pellet. There was a mass of tumor projecting slightly above the parietal surface of the right cerebral hemisphere and measuring 6 mm. in diameter. This growth was directly continuous with a 10 mm. mass of tumor that contained small foci of hemorrhage

and almost completely displaced the left cerebral hemisphere. The tumor was composed of cells with short processes, resembling astrocytes, and elongated spindle-shaped cells resembling spongioblasts. In most places the astrocytes predominated. Large foci of necrosis with palisading of the surrounding cells appeared in many parts of the tumor. Mitotic figures and multinucleated neoplastic giant cells were frequently seen. A phosphotungstic acid stain revealed a few blue staining glial fibrils between the cells, and short fibrillary processes on those cells that resembled astrocytes.

Rat No. 70C.—This rat died 488 days after implantation of the pellet. A soft, gray-white tumor measuring 3 mm. in diameter was seen on the surface of the right cerebral hemisphere, about one-half of which it had replaced. It was composed of cells having a variety of sizes and shapes (Fig. 1). In some areas they were spindle-shaped, while in other parts the cells occasionally possessed 3 or 4 short processes. These latter resembled astrocytes. Mitotic figures, large neoplastic giant cells, and large foci of necrosis and hemorrhage were numerous. A section stained with phosphotungstic acid hematoxylin revealed no intercellular collagen or fibrils. Short fibrillary processes were demonstrated on many of the cells.

Rat No. 59C.—The animal died 553 days after implantation of the pellet. The pellet was surrounded by a 3 mm. zone of gray-white, soft tumor at its inferior end. The predominant cell was large, round, or slightly oval, with a pale eosinophilic cytoplasm that in many instances contained clear vacuoles of moderate size. The nuclei were large, with moderate amounts of clumped, basophilic chromatin. Intermingled with this type of cell were many smaller, round cells of varying size without processes or protoplasmic extensions. Mitotic figures, in certain instances atypical, and large multinucleated neoplastic giant cells were frequently noted. The phosphotungstic acid hematoxylin stain revealed no intercellular or extracellular fibrils or collagen.

All 6 tumors exhibited many of the gross and histologic characteristics of the spongioblastoma multiforme of the human brain. They all arose deep in the cerebral hemisphere and were soft, infiltrating growths that frequently contained large foci of necrosis and hemorrhage. Many different types of cells were seen in the same tumor, and palisading of spongioblasts around the foci of necrosis was frequently noted. Most interesting was the finding of eosinophilic inclusion bodies in the neoplastic giant cells in the tumor of

TABLE II: SURVIVAL TIME FOR THE TWO TYPES OF TUMORS AND THEIR LOCATION

FOURTEEN TUMORS DERIVED FROM NERVOUS TISSUE: AVERAGE SURVIVAL TIME, 299 DAYS

Rat No.*	Sex	Period of survival	Type of tumor	Location of tumor
19C	♂	153	Tumor composed of nerve cells	Intracerebral
27D	♀	194	Astrocytoma	"
52D	♀	206	Spongioblastoma multiforme	"
43D	♀	227	Astrocytoma	"
50D	♀	228	Glioma: unclassified	"
60D	♀	237	Spongioblastoma multiforme	"
71C	♀	278	Tumor composed of nerve cells	" and extracerebral
73C	♀	303	Spongioblastoma multiforme	" lateral ventricle: leptomeninges
43C	♀	303	Glioma: unclassified	"
21C	♀	321	Spongioblastoma multiforme	" leptomeninges
80C	♀	360	Glioma: unclassified	"
20D	♀	366	Oligodendroglioma	"
70C	♀	488	Spongioblastoma multiforme	" "
59C	♀	553	" "	"

TEN TUMORS DERIVED FROM CONNECTIVE TISSUE: AVERAGE SURVIVAL TIME, 282 DAYS

27D	♀	194	Fibrosarcoma	Intracerebral and extracerebral
50D	♀	228	"	Extracerebral and extracranial
24D	♀	247	"	Intracerebral and extracerebral
14C	♂	260	"	" " "
57C	♀	264	"	"
48D	♀	268	"	" " "
43C	♀	303	"	" " "
28D	♀	352	"	" " "
69C	♀	432	"	"
40D	♀	435	"	"

* The letter D appearing after the number of the rat indicates that it was fed a deficient diet and the letter C indicates the control diet. Details of the composition of the diets are reported elsewhere (13).

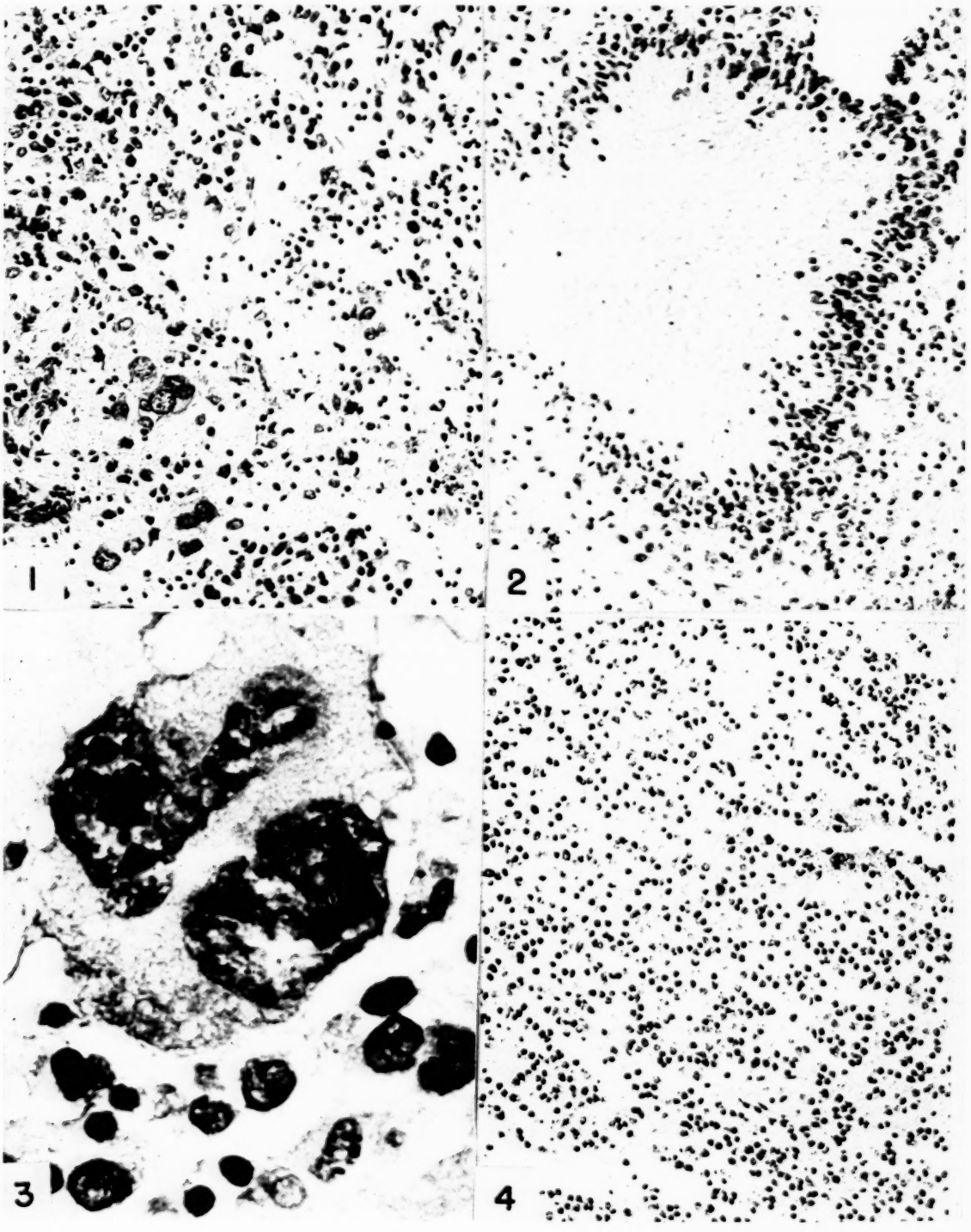


FIG. 1.—Rat No. 70C. Spongioblastoma multiforme showing multinucleated giant cells, spongioblasts, and other types of glial cells. Hematoxylin and eosin stain. Mag. × 160.

FIG. 2.—Rat No. 52D. Spongioblastoma multiforme showing pseudopalisading of spongioblasts surrounding a focus of necrosis. Hematoxylin and eosin stain. Mag. × 160.

FIG. 3.—Rat No. 73C. Neoplastic giant cell in spongioblastoma multiforme. Note lobulated spheroid inclusions in the nucleus. Hematoxylin and eosin stain. Mag. × 1,400.

FIG. 4.—Rat No. 27D. Astrocytoma with cells showing no definite arrangement or pattern. Hematoxylin and eosin stain. Mag. × 160.

Rat No. 73C (Fig. 3). These resembled the type of inclusion seen in the nuclei of neoplastic giant cells in some examples of spongioblastoma multiforme in the human subject. All the tumors gave the characteristic staining reactions of glial tumors with phosphotungstic acid hematoxylin.

Astrocytoma

Rat No. 27D.—This animal died 194 days after implantation of the pellet. A raised nodule 2 mm. in diameter was seen on the superior parietal surface of the right cerebral hemisphere, and a 1 mm. zone of tumor surrounded the pellet. The tumor that projected from the surface of the brain was a fibrosarcoma, composed of pleomorphic spindle-shaped cells that frequently contained mitotic figures. Continuous with, but sharply differentiated from, this tumor was a second type of neoplasm, surrounding the pellet. This was composed of round cells, closely packed together and having a pale, eosinophilic cytoplasm that frequently formed small protoplasmic processes (Fig. 4). The nuclei were round and moderately chromatic. The tumor diffusely infiltrated the brain. Mitotic figures were present, although infrequent, but no neoplastic giant cells were noted. A phosphotungstic acid hematoxylin stain disclosed a moderate amount of brownish-red collagen between the cells in the fibrosarcoma. With this stain many of the cells in the tumor surrounding the pellet showed short, blue, fibrillary processes, and occasionally there were fine, blue fibrils between the cells, but never any collagen.

Rat No. 43D.—This rat lived for 227 days after implantation of the pellet. A small, gray-white focus of discoloration was noted on one side of the pellet, which on histologic examination proved to be a cellular tumor. It was composed of cells resembling astrocytes, with round or slightly oval, moderately chromatic nuclei, and pale eosinophilic cytoplasm. Many of the cells had several short fibrillary processes that were well shown in a phosphotungstic acid hematoxylin stain, which demonstrated also a moderate number of blue staining intercellular fibrils. Mitotic figures were present, although infrequent, but no neoplastic giant cells.

Both these tumors were composed of a single type of glial cell that in most instances were remarkably like astrocytes. The tumors were similar in many respects to the astrocytoma occurring spontaneously in man.

Oligodendroglioma

Rat No. 20D.—This rat died 366 days after implantation of the pellet. A 3 mm. mass of gray-white tumor containing minute red-brown foci of hemorrhage was noted on both sides of the upper half of the pellet. The neoplasm was composed of closely packed, round cells with centrally placed nuclei and pale, clear cytoplasm that gave the cell a perinuclear halo (Fig. 7). There was no pleomorphism, and only this one type of cell was seen. Mitotic figures were frequently observed, but no neoplastic giant cells. There were small foci of necrosis and hemorrhage. No intracellular or intercellular fibrils were brought out by the phosphotungstic acid hematoxylin stain.

The cells in this tumor were typical oligodendral cells. Both the cells and their arrangement were typical of the oligodendroglioma that occurs spontaneously in man, although the necrosis and hemorrhage observed are not seen in the human tumor.

Unclassified Glial Tumors

Rat No. 50D.—This rat died 228 days after implantation of the pellet. There was a nodule of firm, gray-white tumor attached to the outer surface of the calvarium in the right parietal region that was 9 mm. in diameter and 3 mm. deep. It was continuous through the burr hole in the calvarium with a second tumor that extended into the right cerebral hemisphere. The tumor in the hemisphere measured 7 mm. in diameter, and surrounded the superior part of the pellet. The growth involving the calvarium and the cerebral hemispheres was a fibrosarcoma, composed of closely packed, elongated cells, usually arranged in a parallel formation and frequently containing mitotic figures.

At the base of the pellet, but not in contact with the tumor just described, was a small focus of cellular tumor unlike the fibrosarcoma. The cells in this tumor were polyhedral, and some had one or two short processes; the cytoplasm was clear and eosinophilic, and the nuclei were large, irregular, and hyperchromatic. Mitotic figures and neoplastic giant cells were occasionally seen. There were no intercellular or intracellular fibrils or intercellular collagen in this tumor in the section stained with phosphotungstic acid hematoxylin, although large amounts of brown-staining collagen and intracellular fibroglia were present in the fibrosarcoma.

Rat No. 43C.—This rat lived 303 days after implantation of the pellet. When the calvarium was removed the bone over the right cerebral hemisphere was found adherent to the scar produced in the brain by implantation of the pellet. A zone of soft, gray-white tissue, 2 mm. thick and containing small brown-red foci of hemorrhage sharply outlined from the surrounding brain, was seen on the under surface of the pellet. The tumor attached to the calvarium was a fibrosarcoma, composed of spindle-shaped cells containing mitotic figures and of neoplastic giant cells; it filled the subjacent subarachnoidal space and extended into the

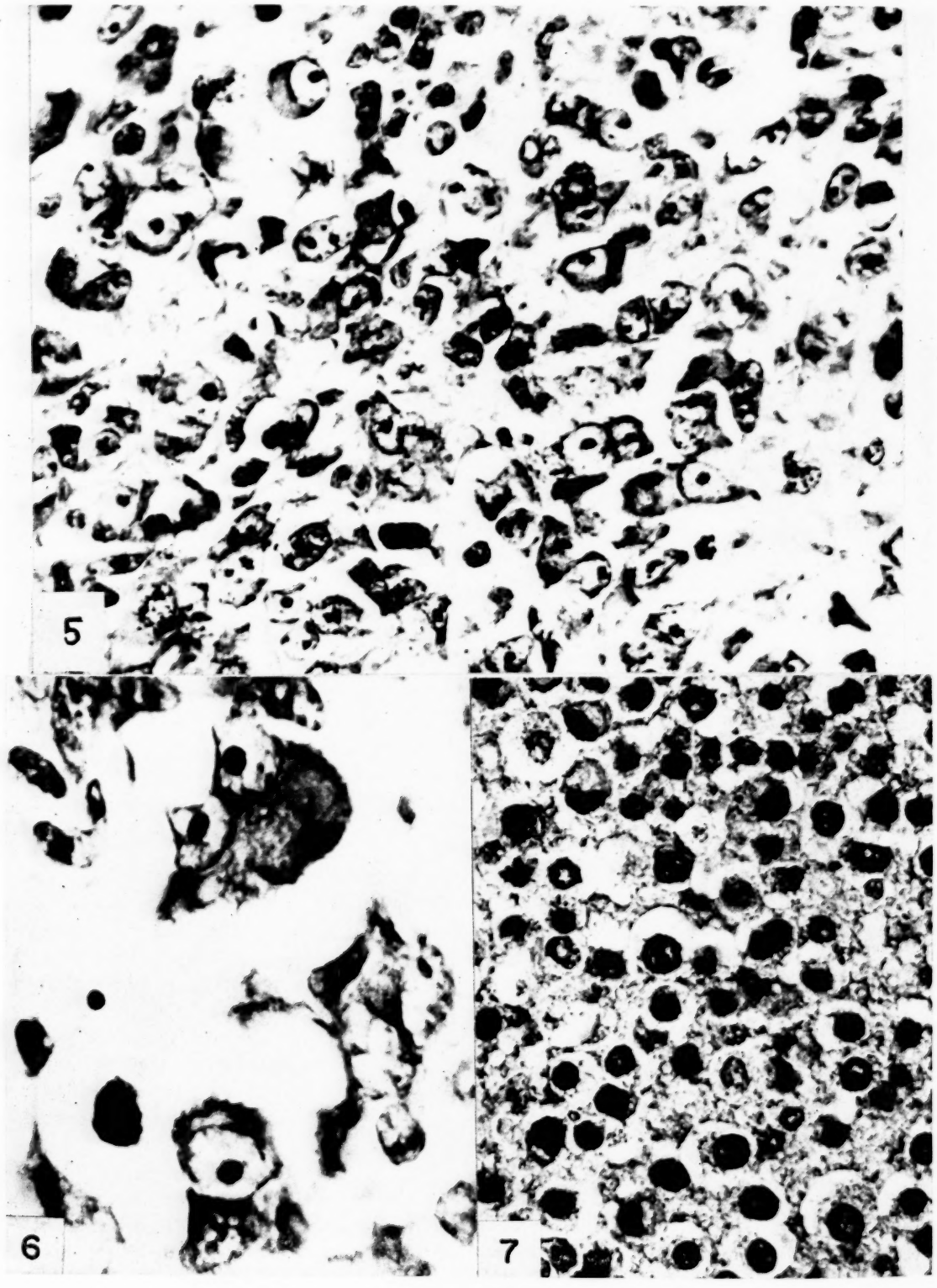


FIG. 5.—Rat No. 19D. Tumor in subarachnoidal space over pellet. Note closely packed stellate cells resembling nerve cells, with round or oval nuclei containing a prominent round nucleolus. Thionin stain for Nissl substance. Mag. × 680.

FIG. 6.—Higher power magnification of nodule shown in Fig. 5, to show stellate cells. Two resembling nerve cells are shown. Note double nucleus in cell in upper part of field, and masses of basophilic material in cytoplasm. Thionin stain for Nissl substance. Mag. × 1,400.

FIG. 7.—Rat No. 20D. Oligodendroglioma. Note characteristic perinuclear halo with small, round, chromatic nucleus. Hematoxylin and eosin stain. Mag. × 680.

small depressed scar on the surface of the brain produced by the insertion of the pellet. The tumor surrounding the lower third of the pellet was composed of closely packed, polygonal cells with scanty cytoplasm and round, deeply basophilic nuclei. In one part of this tumor the cells were more pleomorphic and in many instances showed well developed unipolar and bipolar processes. Blue intracellular fibrils were seen in some of these cells when stained with phosphotungstic acid hematoxylin. Mitotic figures were seen but infrequently, and several large, multinucleated, neoplastic giant cells were noted. Small foci of necrosis with hemorrhage, but without palisading of the surrounding cells, appeared in the tumor.

Rat No. 80C.—This animal died 360 days after implantation of the pellet. Surrounding the inferior end of the pellet there was a small zone of gray-white tumor measuring approximately 1 mm. in diameter. Its cells were round or polygonal, with round, moderately chromatic nuclei, and they diffusely infiltrated the surrounding brain. Small foci of necrosis appeared in the tumor, and mitotic figures were frequent. Small masses of tumor cells were noted in the lateral ventricles. There were no intercellular or intracellular fibrils, nor was there any intercellular collagen present in the section stained with phosphotungstic acid hematoxylin.

All 3 of these tumors were satisfactorily identified as glial tumors by their staining reaction with phosphotungstic acid hematoxylin, but they were not sufficiently characteristic of any of the classified types of glioma to warrant a definite diagnosis. The tumor in rat No. 50D and that in No. 43C had many characteristics of spongioblastoma multiforme, and the growth in rat No. 80C contained cells resembling medulloblasts.

Tumors Composed of Large Numbers of Well Differentiated Nerve Cells

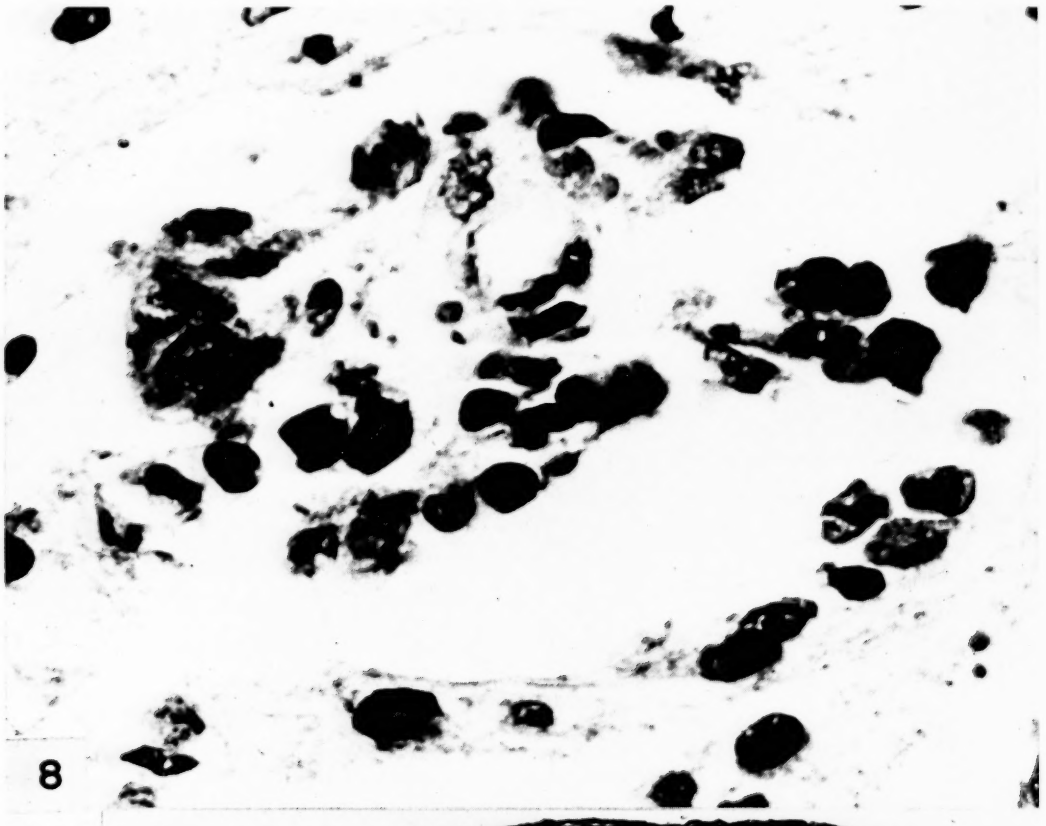
Rat No. 19D.—This animal died 153 days after implantation of the pellet. There was noted on the medial side of the pellet a 1 mm. zone of gray-white tumor. Histologic study showed that this was irregularly outlined against the surrounding nervous tissue, and in several places small, round foci of tumor cells appeared in the adjacent nervous tissue without apparent connection with the main mass of the neoplasm. The subarachnoidal space over the pellet was filled with tumor. The predominant cell was a moderately large, stellate element with a pale eosinophilic cytoplasm that usually formed 2 to 4 short, pointed processes. The nucleus was round, or slightly oval, with scanty chromatin most heavily concentrated at the nuclear membrane and a prominent, round nucleolus (Fig. 5). Occasionally 2 nuclei of this type were seen within a single cell (Fig. 6). Cells of this particular type, resembling nerve cells, usually were seen in large collections closely packed together. Two such large collections were noted in the subarachnoidal space and several others in the tumor along the side of the pellet.

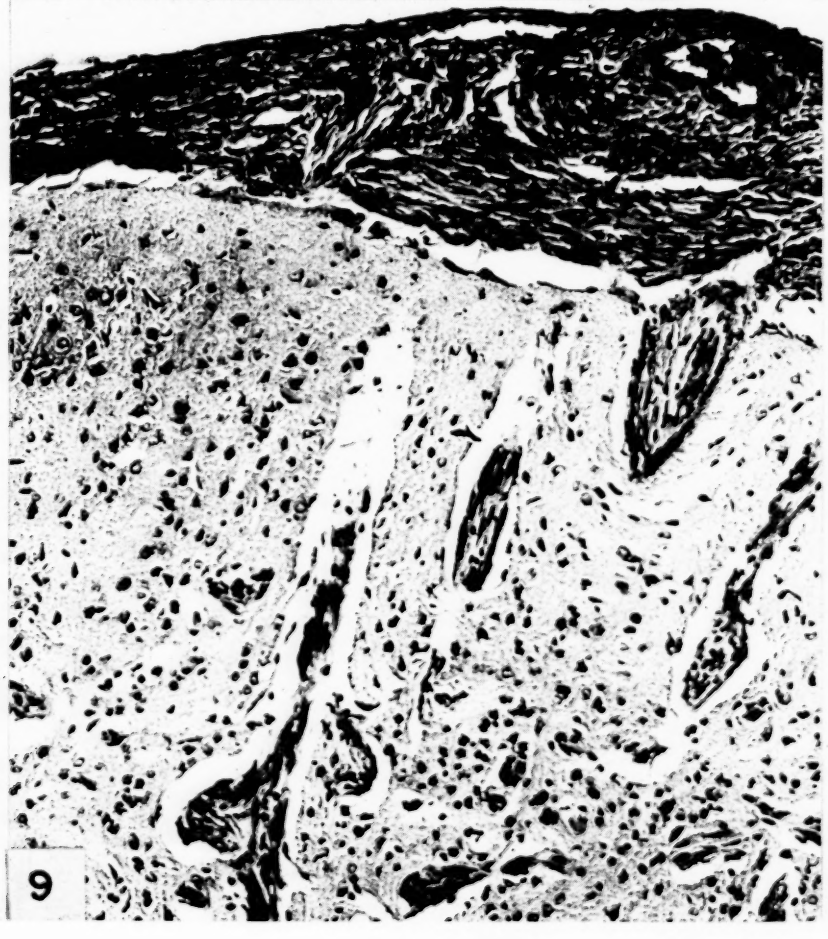
Intermingled with the stellate cells and surrounding the large collections of these cells were smaller cells, varying from a size nearly equal to that of the stellate cell down to approximately the dimensions of a small lymphocyte. The smaller cells, in contrast to the stellate elements, rarely showed any processes but were round, with a small amount of pale, eosinophilic cytoplasm. The nuclei were more chromatic, and rarely contained the round, basophilic nucleolus seen in the stellate cells. Mitotic figures were occasionally seen in both the stellate and the smaller cells. Neoplastic giant cells were not observed, although occasionally one of the stellate cells contained a large, atypical, chromatic nucleus without the characteristic nucleolus.

In a section stained with phosphotungstic acid hematoxylin the processes of the stellate cells were clearly apparent, and in certain instances were observed to extend approximately one-third the width of the cell beyond the cell body. Where the stellate cells were closely packed together in the subarachnoidal space, there was no discernible intercellular substance. In the other parts of the tumor, composed mainly of the smaller cells, there was no intercellular material except in one moderately large focus, where sparsely arranged small cells interspersed with an occasional stellate cell were surrounded by bands of brown-staining collagen, which broke up the collections of cells into small isolated groups.

In a section stained with thionin to demonstrate Nissl substance the cytoplasm of the stellate cells contained moderate amounts of basophilic material in large masses; but no small masses resembling Nissl bodies were noted (Fig. 6). In cells with unusually large nuclei the basophilic material was seen only at the periphery, near the cell membrane. The small cells did not contain any of the clumped basophilic substance noted in the stellate cells, but the cytoplasm of this cell was generally basophilic. Sections cut 40 microns thick and prepared by the Bodian method for the demonstration of neurofibrillae revealed argyrophilic fibrils in many of the stellate cells that continued into the cell processes. Argyrophilic fibrils were not seen in the smaller cells.

Rat No. 71C.—This rat died 278 days after implantation of the pellet. There was a round, 3 mm. nodule of tumor projecting approximately 1 mm. above the surface of the brain at the site of the pellet in the right cerebral hemisphere. In coronal sections the tumor noted on the surface of the brain surrounded the pellet





FIGS. 8 AND 9

and almost entirely replaced the superior part of the hemisphere. The growth was composed of many different types of cells showing no characteristic arrangement or growth pattern. Approximately half of the cells were stellate, with 3 or more short projections of cytoplasm from the main cell body. The processes and cytoplasm of these cells was moderately eosinophilic and the nuclei were round or slightly oval, with a heavy accumulation of chromatin at the nuclear membrane and a round and prominent nucleolus; occasionally there were 2 nuclei. These cells were remarkably like nerve cells. The remainder of the cells were smaller, and only occasionally did they show any resemblance to the stellate cells. Generally the small cells were round, with scanty eosinophilic cytoplasm and round, chromatic nuclei whose chromatin showed no particular arrangement. Transition forms were seen between the smaller cells and the stellate cells, elements with 1 or 2 short processes and a nucleus showing evidence of chromatin arrangement similar to that seen in the stellate cells. Mitotic figures were noted in both types of cells, but no neoplastic giant cells were found. Large foci of necrosis occurred in many parts of the tumor. In one place the growth had extended into the lateral ventricle. Invasion of the perivascular spaces was frequent, and in one instance a perivascular space in the surrounding brain not involved by the neoplasm contained a large number of tumor cells. One of these was a large, stellate cell resembling a nerve cell, with 2 characteristic nuclei each containing small round nucleoli (Fig. 8).

A section stained with phosphotungstic acid hematoxylin clearly showed the processes of the stellate cells. No intercellular or intracellular fibrils were noted, nor was any collagen demonstrable between the cells. In a section stained with thionin to show Nissl substance, large masses of chromophilic substance were seen in many of the large stellate cells, and the cytoplasm of the smaller cells was basophilic. With this stain the chromatin in the nucleus was brought out in great detail, and its arrangement resembled the distribution and arrangement seen in nerve cells.

Several sections were prepared by the Bodian method for neurofibrillae, which demonstrated fine argyrophilic fibrils in the cytoplasm and processes of many of the stellate cells. The processes extended 20 to 30 microns from the cell body. No argyrophilic fibers were noted in any of the smaller cells.

The stellate cells found in both these tumors were definitely identified as nerve cells, since their cytoplasm contained a chromophilic substance and argyrophilic fibrils, and they had the characteristic nuclei. It is noteworthy that these cells frequently contained double nuclei, for although double nuclei are never seen in nonneoplastic nerve cells, they are not infrequently observed in the nerve cells of ganglioneuromas in the human subject. The presence in these tumors of less well differentiated cells not at all resembling nerve cells, with transition forms to the stellate cell, indicates that the stellate cells are differentiated from the former type of cell. However, the stellate cells demonstrated all characteristics usually seen in neoplastic cells, including mitotic division and invasion of tissue.

Tumors Derived from Connective Tissue

Extracranial or extracerebral fibrosarcoma (Rat 28D, 24D, 27D, 50D, 48D, 43C, 14C): The tumor in these 7 rats either involved the leptomeninges, the dura mater, or extended through the burrhole to involve the soft tissues of the scalp. In all instances there was a large mass of tumor surrounding the pellet, usually on its superior surface. In those cases where a growth involved the subcutaneous tissues of the scalp it would seem more likely that it grew up through the burrhole and secondarily involved the brain than that it arose in the subcutaneous tissue from small pieces of methylcholanthrene left there at operation.

Intracerebral Fibrosarcoma

Rat 69C, 57C, 40D.—In these 3 rats the tumor arose within the brain and was nowhere in contact with the leptomeninges. The fact that these growths developed entirely within the brain is good evidence that its fibroblastic elements respond in the same general way to carcinogenic stimulation by methylcholanthrene as those of other somatic tissues. The tumor in all 3 brains was sharply circumscribed and demarcated from the adjacent nervous tissue, and was always in close contact with the pellet of methylcholanthrene.

Histologic study of all these growths revealed characteristic fibrosarcoma, composed of spindle-shaped cells that were usually arranged in parallel rows and showed various degrees of anaplasia in different tumors. In some there was considerable pleomorphism,

DESCRIPTION OF FIGURES 8 AND 9

Fig. 8.—Rat No. 71C. Perivascular space of small vein filled with tumor cells. Note stellate cell resembling a nerve cell on left side of perivascular space that contains two nuclei with characteristic prominent round nucleolus. Hematoxylin and eosin stain. Mag. × 1,000.

Fig. 9.—Rat No. 50D. Fibrosarcoma filling subarachnoidal space and extending into perivascular spaces. Hematoxylin and eosin stain. Mag. × 160.

with large numbers of neoplastic giant cells and mitotic figures, while in others neoplastic giant cells were never seen and mitotic figures were infrequent. In all the tumors phosphotungstic acid stains demonstrated fine intracellular fibroglia and varying amounts of brownish-red staining collagen between the cells. The tumors tended to remain sharply outlined from the surrounding nervous tissue and did not infiltrate it diffusely as did those derived from nervous tissue. In many of these sarcomas there was a tendency for the growth to fill the subarachnoidal space and infiltrate perivascular spaces at a considerable distance from the main mass (Fig. 9).

COMMENT

Twenty-one of 42 (50 per cent) rats that survived long enough with pellets of methylcholanthrene in the brain developed intracranial tumors. This figure compares favorably with those for mice with methylcholanthrene reported by Zimmerman and Arnold (46 per cent), and Seligman and Shear (65 per cent); Peers, on the other hand, reported only 32 per cent (28 of 87). It should be noted, however, that Peers used stock albino mice instead of C3H mice, which were employed by Seligman and Shear, and by Zimmerman and Arnold. Apparently the susceptibility of the strain is important, a point that has been emphasized by Arnold and Zimmerman. The incidence of tumors of nervous tissue origin (66 per cent of intracranial tumors) obtained in these experiments with rats is approximately the same as has been reported in mice by Seligman and Shear (84 per cent), Zimmerman and Arnold (52 per cent), and Peers (53 per cent). It is apparent from these figures that the cells of the central nervous system of the rat respond as rapidly to carcinogenic stimulation as those of the mouse if a comparable procedure is employed. Previous failure in the rat can be explained in the same way as failure in the mouse before the work of Seligman and Shear; namely, the carcinogenic agent was either not applied in sufficient concentration or for a long enough period of time.

The small number of rats in the experiments recorded here probably accounts for the failure to observe all the types of glial tumors that have been described in mice. Unusual caution has been exercised in classifying the tumors, and the diagnosis of a definite glial type has not been made unless the growth exhibited most of the characteristics usually observed in the human subject. As a result, it was not possible to classify 3 of the tumors definitely, although it could be said with full certainty that they were of glial origin.

The most unusual type noted concerned the neoplasms of rats 19D and 71C. Both contained large numbers of fairly well differentiated nerve cells, and both exhibited the characteristics of a truly malignant tumor. Neoplasia of nerve cells has not been observed in the intracranial tumors produced in mice. However, tumors arising from the cells of the sympathetic ganglia and composed of neuroblasts are not an infrequent growth in children (19). In many instances these contain considerable numbers of fairly well differentiated nerve cells, and in rare cases all the cells in the tumor have gone on to differentiate into mature nerve cells, presumably of a nonmalignant character (4, 5). Many of these tumors composed of differentiated nerve cells may exhibit all the characteristics of malignant tumors, including metastasis (6, 16). Neoplasia of nerve cells is therefore not a new observation, since it does occur spontaneously in man though it has not been observed before in experimental carcinogenesis.

It is interesting to speculate about the histogenesis of the tumors that were composed for the most part of neoplastic nerve cells. It is generally accepted that the fully matured and differentiated nerve cell represents in all probability the most specialized and highly differentiated cell in the animal organism. It possesses no power to proliferate, a limited power to regenerate its peripheral processes, and is most sensitive to anoxemia and toxic agents in concentrations that do not affect other somatic cells. For these reasons neoplasia induced by a carcinogenic agent acting directly on the mature nerve cell appears highly improbable. A more plausible explanation would be that the carcinogen elicited neoplasia of some primitive cell in the brain that possesses the potentialities of differentiating into nerve cells. Presumably the tumor cells that resembled nerve cells represented a further differentiation of these elements, since not all the cells in the tumor were recognizable as nerve cells. Moreover, those showing the best differentiation in certain instances appeared in discrete foci.

SUMMARY

Pellets of methylcholanthrene in 30 per cent concentration fused with chemically pure cholesterol implanted into the right cerebral hemisphere of 42 albino rats of the Rockland strain of both sexes produced intracranial tumors in 21 of the animals. Fourteen of the 21 rats developed growths derived from nervous tissue, and 10 developed fibrosarcomas; 3 animals had both types. The following types of tumor derived from nervous tissue were produced: 6 spongioblastoma multiforme, 2 astrocytomas, 1 oligodendroglioma, and 2 tumors that for the most part were composed of large numbers of fairly well differentiated nerve cells. The 2 last named are the first reported instances of induced neoplasia of nerve cells. It was not possible to classify 3 of the tumors derived from nervous tissue.

There was no significant difference in the survival time of the rats with neoplasms derived from nervous tissue and those with fibrosarcomas.

The tumors derived from nervous tissue were easily differentiated macroscopically from the fibrosarcomas. The former were soft, infiltrating, frequently contained foci of hemorrhage, and were poorly demarcated from the surrounding brain. The latter were sharply circumscribed, firm, and rarely contained hemorrhagic foci.

These experiments demonstrate that the cells of the central nervous system of the rat respond to carcinogenic stimulation as readily as those of the mouse if a high concentration of carcinogen is kept in contact with the nervous tissue for a sufficient length of time.

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The Response of the Central Nervous System of the Rat to Methylcholanthrene

II. The Effect of a Diet Deficient in Thiamine and Riboflavin on the Induction of Tumors Derived from Nervous Tissue*

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INTRODUCTION

The work of Kinosita (7), demonstrating a dietary factor in the production of carcinoma of the liver in rats with *p*-dimethylaminoazobenzene, and that of Kensler, Sugiura, Young, Halter, and Rhoads (6), who showed that riboflavin and casein can protect against this compound, suggested the possibility of influencing similarly the induction of tumors in nervous tissue by methylcholanthrene. Previous work, indicating that the cells of the central nervous system of the rat are resistant to carcinogenic stimulation (12, 15, 19, 22), offered an additional impetus to determine if this could be enhanced by altering intracellular metabolism through vitamin deficiency.

The following communication reports the effect of periodic removal of thiamine and riboflavin from the diet [1] of rats with pellets of 30 per cent methylcholanthrene implanted in their brains. Thiamine and riboflavin were selected for study because these substances are known to be concerned with enzyme systems and intracellular metabolism (3); moreover, a deficiency of either produces pathologic change in nervous tissue (17, 18).

The morphologic and histologic study of the tumors that were induced are reported elsewhere (14).

MATERIAL

Eighty-five young male or female rats of the Rockland strain, about 3 months of age and averaging approximately 120 grams in weight, were employed. There were 55 females and only 30 males, because of the greater availability of female animals from the breeding colony in the Department of Pathology. Young growing animals were selected, rather than adults, so that the effect of the vitamin deficiency could be more readily observed in the weight curves (Fig. 1).

Pellets of 30 per cent methylcholanthrene fused with chemically pure cholesterol, each weighing approximately 25 mgm., were implanted in the right cerebral hemisphere. The details of making the pellets and the operation for implanting them, as well as the necropsy and histologic technic employed for diagnosis of the tumors, have been described elsewhere (14).

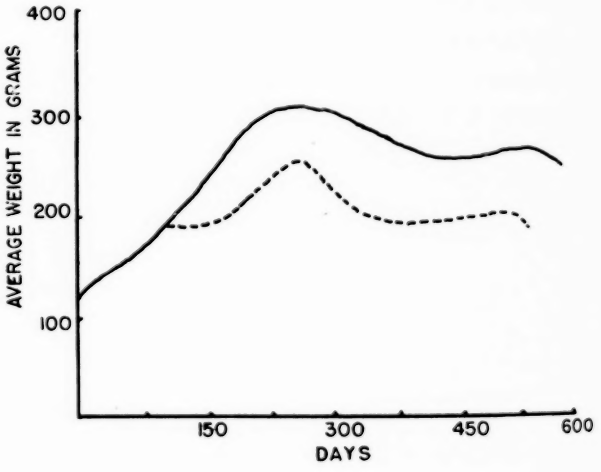

FIG. 1

All the animals were housed in large wire cages, with wire bottoms that allowed the feces to fall out of their reach. The sexes were separated, and not more than 15 rats were placed in any one cage. The cages were cleaned weekly. Forty-three days after the pellets of methylcholanthrene had been implanted, and all rats with infected operation wounds had recovered, the animals were grouped for the experimental diets. Thirty were placed in the deficient group and 22 in the control group. Because the diets employed were completely synthetic, and the animals

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[1] Niacin was also removed, but since the rat can synthesize this substance its elimination from the diet is probably not significant.

would have to be maintained on them for a long period of time, it was thought advisable to place more rats in the deficient group, since unavoidable losses in this group would probably be heavier than in the control groups that received adequate amounts of vitamins.

The stock formula used for the incomplete preparation of the complete and deficient diets was:

Corn starch	73.0%
Commercial casein *	16.0
Salt mixture No. 185, McCollum's	5.0
Cod liver oil (U.S.P.)	5.5
Wheat germ oil	0.5

* Thoroughly washed with acidulated water.

The complete diet, for the control rats, was made by adding the following amounts of synthetic vitamins [2] to 100 gm. of the mixture above:

Thiamine	0.75 mgm.
Riboflavin	1.1 "
Niacin	9.4 "
Pyridoxine	1.13 "
Pantothenic acid	2.82 "
Choline	94.0 "

The deficient diet for the experimental rats contained all the ingredients given alone for the complete diet except thiamine, riboflavin, and niacin.

The diets were prepared by mixing the corn starch, casein, salt mixture, cod liver oil, and wheat germ oil with water to a thick consistency, and adding appropriate amounts of the synthetic vitamins dissolved in water. When prepared, the mixtures were kept in a refrigerator and fed daily to the animals in small dishes. Fresh diet was prepared every 3 days to avoid possible contamination of the food by molds.

EXPERIMENTAL PROCEDURE

Following a 43 day recovery period after the operation the rats were placed on the diets. After 5 weeks those receiving the deficient diet failed to gain as rapidly as those given the complete diet, appeared less active, had ruffled and poorly kept fur, and were irritable when handled. Following a 3 week period in which the animals on the deficient diet failed to gain weight comparably with the controls, one drop of a solution containing approximately 18 μgm. of thiamine, 45 μgm. of riboflavin, and 18 μgm. of niacin was given orally to each rat for 2 days. Following the administration of these vitamins the animals promptly gained weight and their general physical appearance improved remarkably. The improvement generally lasted for 3 to 4 weeks, when the same symptoms reappeared and the average gain of weight again was reduced and finally converted into a loss. This routine of successive periods of deficiency was successfully carried out for 435 days, when the last rat in the deficient group died. During the course of the experiment the control rats gained on an average about 75 gm. less per animal than the deficient ones (Fig. 1). Both the deficient and control groups of rats attained their maximal growth and weight approximately 227 days after the experiment was started. This weight was maintained as a well defined plateau for about 70 days, when both groups simultaneously lost an average of approximately 60 gm.; this second level was maintained for the remainder of the experiment. The loss of weight noted after the plateau had been maintained for 70 days was probably the result of some other deficiency factor, since both groups showed a nearly identical response.

[2] Supplied through the courtesy of Merck and Company.

RESULTS

Twenty-one of the 42 rats that survived past the time of appearance of the first tumor developed an intracranial neoplasm. The incidence in the deficient rats as compared to the controls was approximately the same: 46 per cent (11 of 24) in the deficient group and 50 per cent (9 of 18) in the controls. Fourteen of the rats developed tumors derived from nervous tissue, and 10 had tumors derived from connective tissue. Three had both types.

The incidence of tumors derived from nervous tissue was nearly the same in the deficient and control groups: 39 per cent (7 of 18) in the controls, and 35 per cent (7 of 20) in the deficient group. The tumors derived from connective tissue likewise showed no significant difference in their incidence in the two groups. Twenty-five per cent (6 of 24) of the rats in the deficient group developed this type of tumor and 22 per cent (4 of 18) of the controls.

The induction period for tumors derived from nervous tissue was notably less in the deficient rats than in the controls. Six of those derived from nervous tissue arose in the deficient group before any developed in the rats receiving the control diet (Table I). Moreover, there was an interval of 41 days between the time of appearance of the last of the first 6 tumors derived from nervous tissue in the deficient rats, and the appearance of the first tumor from nervous tissue in the control group. Only 1 other growth derived from nervous tissue appeared in the deficient group, and that was late in the experiment, the induction period in this rat having been 366 days. The average induction period for this type of neoplasm was 230 days for the rats receiving the deficient diet, and 372 days for those on the control diet. The average difference in the induction period for tumors derived from nervous tissue was 142 days.

There was no significant difference in the length

of the induction period for tumors derived from connective tissue. The first 2 fibrosarcomas developed in deficient rats that also developed a tumor from nervous tissue, but 32 days later a fibrosarcoma developed in a control rat, and for the remainder of the experiment this type of tumor appeared as frequently in the deficient rats as in the controls (Table II). The average induction period for the fibrosarcomas was 292 days in the deficient rats and 315 days in the controls. The average difference in the induction period for tumors derived from connective tissue was 23 days.

TABLE I: TUMORS DERIVED FROM NERVOUS TISSUE ARRANGED IN ORDER OF APPEARANCE

Necropsy no.	Experimental period, days	Deficient rats	Control rats
19D	153	+	
27D	194	+*	
52D	206	+	
43D	227	+	
50D	228	+*	
60D	237	+	
71C	278		+
43C	303		+*
73C	303		+
21C	321		+
80C	360		+
20D	366	+	
70C	488		+
59C	553		+

* Indicates rat also developed a fibrosarcoma.

The number of animals in these experiments is too small to justify a detailed statistical analysis. It should be noted, however, that while the average difference of 23 days in the induction period for tumors derived from connective tissue is statistically insignificant, there is a high probability that the average difference of 142 days for tumors derived from nervous tissue may prove statistically reliable if the number of observations is increased.

COMMENT

The results of these experiments indicate that vitamin deficiency in rats significantly altered the susceptibility of the cells of the nervous system to the carcinogenic action of methylcholanthrene, while the connective tissue cells were apparently unaffected. The development of fibrosarcomas, which was not affected by the deficiency, actually afforded a control experiment to emphasize further the observed effect of the deficiency on the origin of tumors from nervous tissue. This result is noteworthy in view of the known effects of thiamine (18) and riboflavin (17) on nervous tissue, and the apparent indifference of connective tissue elements to a deficiency of these vitamins. It is impossible to say at this time without additional experimental work which deficiency altered the susceptibility of the nervous tissue to the action of the carcinogen. The statement can be made, however, that certain processes of intracellular oxidation of the nervous system were probably altered significantly as a result of the removal of thiamine and riboflavin from the diet.

Mention should be made here of the fact that niacin also was removed from the deficient diet. It is most unlikely, however, that its absence produced any significant alteration in the intracellular metabolism of the nervous tissue, since the rat apparently can synthesize this substance and hence does not develop the signs of deficiency noted in other animals when niacin is withheld (2, 16). Nevertheless, it was decided to remove niacin from the diet so that the amount of this vitamin in the tissues would be restricted at least as far as possible. Moreover, there is reason to believe that thiamine, riboflavin, and niacin are all collectively concerned with biological oxidation, and that any investigation of one demands consideration of the other two (1).

TABLE II: TUMORS DERIVED FROM CONNECTIVE TISSUE ARRANGED IN ORDER OF APPEARANCE

Necropsy no.	Experimental period, days	Deficient rats	Control rats
27D	194	+*	
50D	228	+*	
14C	260		+
57C	264		+
48D	268	+	
24D	274	+	
43C	303		+*
28D	352	+	
69C	432		+
40D	435	+	

* Indicates rat also developed a tumor derived from nervous tissue.

The thiamine deficiency in the rats of this experiment interfered with the normal oxidation of carbohydrate by the cells of the nervous system that is so vitally essential to their metabolism and function. It has been demonstrated that a phosphoric acid ester of thiamine acts as a coenzyme necessary for the oxidation of pyruvic acid, an intermediate in carbohydrate oxidation. In thiamine deficiency there is first a decrease in free thiamine in the tissue and then a decrease in the phosphoric acid ester of thiamine (cocarboxylase). As a result of this decrease in cocarboxylase, pyruvic acid accumulates in the tissues and oxidative mechanisms in the brain are inhibited. Although less well understood, it has been suggested that the symptoms of thiamine deficiency may be correlated with a decreased acetylcholine content in certain tissues of

the body (3), since it has been demonstrated that thiamine inhibits the action of cholinesterase (4). The work of Zeller and Birkhäuser (21) would indicate, however, that alteration of this enzyme probably is not a factor concerned in thiamine deficiency of nervous tissue, since they demonstrated that cholinesterase is not reduced in the brain in thiamine deficient rats when significant reductions of this enzyme are demonstrable in the liver.

The deficiency of riboflavin produced in the rats of this experiment would appear significant, since this substance is related to more enzyme systems than any of the known vitamins and plays an important role in the intracellular oxidative mechanisms of the entire animal organism (3). The possibility that the riboflavin deficiency may have been responsible for the results observed in these experiments is suggested by the work of Kensler, Sugiura, Young, Halter, and Rhoads (6), who have demonstrated that this substance can protect the liver of rats fed *p*-dimethylaminoazobenzene from developing cancer. Kensler, Sugiura, and Rhoads (5) have shown that both riboflavin and coenzyme I were decreased in the livers of rats fed *p*-dimethylaminoazobenzene presumably as the result of a toxic metabolite produced by the carcinogen. It has been further demonstrated that the production of cancer in the liver paralleled the inhibition of the coenzyme I system (13). Since the niacin-containing coenzyme (coenzyme I) functions in biologic oxidations by the transfer of electrons to riboflavin enzymes, this observation suggested a niacin deficiency. Because the rat can synthesize niacin, and the addition of large amounts of this substance to the diet failed to prevent the lowered coenzyme I content of the liver, it was concluded that the lack of riboflavin interfered in some way with the normal mechanism of the body that protects against the carcinogenic effect of the *p*-dimethylaminoazobenzene, the interference causing the formation of an abnormal metabolite that prevented the synthesis of coenzyme I. It was further demonstrated that the toxic metabolite was harmful to normal liver cells, but had no effect upon hepatic tumor cells. From these observations arose a new idea on the genesis of the neoplasia effected in liver cells by *p*-dimethylaminoazobenzene: The liver cells were forced to develop a new system of intracellular oxidation not dependent upon either riboflavin or niacin and not interfered with by the toxic metabolite. This new method of respiration apparently represented an irreversible change, which might be the mutation said to be present in malignant tissue.

It is difficult to imagine any parallel mechanism at play between the production of intracranial tumors in the experiments reported here and experimental hepatic tumors produced with *p*-dimethylaminoazobenzene, since an adequate diet failed to protect the control rats from developing intracranial tumors. However, there was a common factor of riboflavin deficiency in both cases. All that it is possible to say now of the intracranial tumors is that the deficiency so altered the nervous tissue that it responded sooner to the carcinogenic stimulation of the methylcholanthrene. If there was a reorganized cellular metabolism in response to a conditioned deficiency produced by the carcinogen such as has been suggested for the hepatic tumors, and it accounts for neoplasia of the cells of the nervous system in these experiments, it must be assumed that the new systems of metabolism were induced by factors other than the dietary factor, which was not present in the control rats, where there was the same tumor incidence.

These experiments are open to criticism because there was no group of animals to control the inanition effect produced by the deficient diet. It was originally planned to run such a control group, but after the pellets had been implanted, and before the experimental groups were selected, it was necessary to kill a significant number of animals because they had developed "middle ear disease." It was then decided that it would be better to have more animals in two larger groups than to divide them into three small groups.

Generally, however, investigation has shown that underfeeding and inanition lengthen the period of induction for tumors induced with carcinogenic agents. Tannenbaum (2), studying the effect of inanition on the induction and growth of spontaneous breast and lung tumors, and skin tumors induced with benzpyrene, reported that fewer tumors developed, and at a later period, in underfed animals than in those receiving an adequate diet. The rate of growth of the tumor once it was initiated in the underfed animal was the same in both the spontaneous and the induced tumors. McCay, Ellis, Barnes, Smith, and Sperling (11) have observed a lower incidence of spontaneous tumors in rats with retarded growth. Lavik and Baumann (8), studying the effect of diet on the incidence of tumors induced with methylcholanthrene in the skin of mice, reported an increased number of tumors with a high fat diet. In another study these authors (9) observed that the animals eating the high fat diet consumed more calories, and that the increased caloric intake correlated roughly with the rate of tumor production. It would seem fair to conclude from these experiments concerned with inanition that the uncontrolled inanition factor in the experiments reported here would not be important, since the influence of inanition and a lowered caloric intake would have tended to lengthen the induction period rather than to decrease it.

SUMMARY

Under the conditions of the experiment, the periodic removal of thiamine and riboflavin from the diet of white rats with intracerebral pellets of methylcholanthrene produced a significant reduction in the length of the induction period of tumors derived from nervous tissue. Their average induction time was 230 days for the rats given the deficient diet, and 372 days for those fed the control diet. The incidence of tumors derived from nervous tissue was the same on both deficient and adequate diets. The deficiency had no effect upon either the incidence or the length of the induction period for tumors derived from connective tissue.

It is suggested that the altered metabolism of the cells of the nervous system resulting from the deficiency of thiamine and riboflavin caused the cells to respond more readily to the carcinogen. It is not possible to say which deficiency was responsible for the altered susceptibility of the nervous tissue.

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The Determination of *p*-Dimethylaminoazobenzene, *p*-Monomethylaminoazobenzene, and *p*-Aminoazobenzene in Tissue*

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An understanding of the mechanism by which liver tumors are produced when *p*-dimethylaminoazobenzene is fed to rats might be hastened considerably if more complete information were available on the fate of this substance in the animal. Such a study, however, necessarily must await suitable analytical procedures for the quantitative determination not only of *p*-dimethylaminoazobenzene itself but also of closely related physiological degradation products. The present communication deals with the determination of small amounts of *p*-dimethylaminoazobenzene, *p*-monomethylaminoazobenzene, and *p*-aminoazobenzene in tissue.

GENERAL CONSIDERATIONS

These azo dyes possess the following pertinent chemical characteristics. They are stable to digestion with cold alcoholic KOH in the presence of tissue. They can be quantitatively extracted from such mixtures with petroleum ether and, unlike the majority of unsaponifiable substances, they can then be quantitatively extracted from the petroleum ether solution into strong HCl to yield red to orange solutions. These solutions obey Beer's law and have extinction coefficients of sufficient magnitude for the accurate estimation of 0.5 μgm. of each azo dye. The dyes may be extracted back into petroleum ether after the acid solution is made alkaline, to yield solutions free from other unsaponifiable matter. Petroleum ether solutions of mixtures of these three compounds can be chromatographed on alumina to yield three bands, which can be separately eluted with benzene in the order *p*-dimethylaminoazobenzene > *p*-monomethylaminoazobenzene > *p*-aminoazobenzene. Each dye may then be quantitatively extracted into acid and estimated colorimetrically. The presence of moderate amounts of tissue in the original digestion does not interfere with any of these manipulations. All these operations can be successfully performed with as little as 1 to 5 μgm. of each azo dye.

The color intensities of many azo dyes vary somewhat with the concentration of the HCl solutions employed. The color intensities of the three dyes under consideration were found to be maximal in 7 *N* HCl, the concentration chosen for routine work. The intensities diminish slowly below 6 *N*, and more quickly above 8 *N*. Above 8 *N* the dyes are slowly destroyed at room temperature; in 7 *N* acid the dyes are stable for at least 24 hours.

REAGENTS

1. Petroleum ether. Redistilled Skelly Solve B (b.p., 66-68° C.).
2. 95 per cent ethanol.
3. Benzene.
4. 11 *N* KOH. Equal weights of 85% U.S.P. KOH and water.
5. 7 *N* HCl. Approximately 1.4 volumes of concentrated HCl to 1 volume of water. The cold solution titrated to within 0.05 units of 7 *N*.
6. Aluminum oxide. Merck reagent. Since the azo dyes are strongly adsorbed, a very weakly adsorbent alumina was employed. One-hundred mesh alumina was moistened with methanol and then thoroughly dried at 90-100° C. Not all samples of alumina were satisfactory after this treatment, and the reactivation was adjusted so that the final product separated the three dyes as described in the text. Screening or sedimentation was sometimes necessary to remove very fine particles that clogged the narrow adsorption columns employed.
7. Azo compounds. Each azo dye was twice recrystallized from benzene-petroleum ether and each was found to be chromatographically pure under the conditions of adsorption employed. The *p*-dimethylaminoazobenzene was an Eastman product, No. 338, and had a final m.p. of 116.5-117.2° C. corr.; per cent N = 18.40, 18.44 (Theor. = 18.42). The *p*-aminoazobenzene was prepared by rearranging diazoaminoben-

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zene (3) according to the usual directions (2); the final m.p. was 124.5-125.4° C. corr.; per cent N = 21.43, 21.52 (Theor. = 21.32).

The only directions found for the preparation of *p*-monomethylaminoazobenzene were those of Berju (1), who attempted to prepare this compound in 1884 by reacting equimolar amounts of *p*-aminoazobenzene and methyl iodide. By recrystallization of the reaction mixture from alcohol he obtained a compound of m.p. 180°. It is not clear from his description whether this melting point refers to a free base or, as is probable, to a hydroiodide. In an attempt to repeat this work we procured a mixture from which no homogeneous crystalline compound could be obtained. However, after decomposition with Na_2CO_3 a mixture of azo compounds resulted which, when chromatographed on alumina, yielded three bands. The top and bottom bands were shown by mixed m.p. determinations to be *p*-aminoazobenzene and *p*-dimethylaminoazobenzene respectively. The middle band was assumed to be *p*-monomethylaminoazobenzene. This compound had a m.p. of 86-87° C. corr. and yielded an acetyl derivative of m.p. 88-89° C. corr.; a mixture of these two compounds melted at about 50°. Berju's compound of m.p. 180° was stated to yield an acetyl derivative having a m.p. of 139° (the acetyl derivative of *p*-aminoazobenzene melts at 146°). Hence an unequivocal and practical synthesis for *p*-monomethylaminoazobenzene became necessary.

N-methyl diazoaminobenzene was prepared by coupling equimolar amounts of benzenediazonium chloride and N-methyl aniline in the presence of excess sodium acetate. Little direct coupling in the *para-* position occurred, and the diazoamino compound separated as an orange oil in yields of approximately 95 per cent. This compound was then rearranged by heating a mixture of 20 gm. of the dry diazoamino compound with 30 gm. of N-methyl aniline and 10 gm. of N-methyl aniline hydrochloride at 45° C. for 3 hours with frequent stirring. The mixture became dark red, and was allowed to stand at room temperature for 12 hours. At the end of the reaction (no evolution of N_2 with strong acid) the hydrochloride of the *p*-monomethylaminoazobenzene was precipitated with excess 10 per cent HCl and filtered from the mixture. The hydrochloride was decomposed with Na_2CO_3 and the crude base dried and recrystallized from benzene-petroleum ether. Orange tufts of fine needles were obtained, m.p., 88.0-88.5° C. corr.; per cent N = 19.68, 19.80 (Theor. = 19.89); acetyl derivative, m.p., 90-91° C. corr. The yields from the rearrangement were approximately 60 per cent.

This compound gave no melting point depression with the "middle compound" obtained as described from the reaction of *p*-aminoazobenzene and methyl iodide. For the further characterization of the dye it was split by refluxing with an excess of $Na_2S_2O_4$ in 1:1 ethanol-water for 10 minutes. The solution was made alkaline and the amines extracted with ether. Upon removal of solvent in a current of N_2 the amines were suspended in dilute base and steam-distilled in a current of N_2 for the separation of the volatile monoamine from the nonvolatile diamine. Each fraction was then re-extracted with ether. After the removal of the solvent the benzamide of each compound was prepared by the Schotten-Baumann method and each derivative recrystallized twice from ethanol-water. Mixed melting point determinations with the benzamides of the authentic amines showed that the volatile amine was aniline and that the diamine was N-monomethyl-*p*-phenylene diamine.

Apparatus

The apparatus needed is relatively simple. Standard equipment suitable for the following operations on small amounts of material can be used: for grinding tissue, for mixing aqueous solutions with petroleum ether and for removing the ether quantitatively, for chromatographic adsorption, and for the measurement of color intensity. The following were found convenient for rapid routine determinations:

1. Evelyn photoelectric colorimeter with filters 500 and 520 and selected "S" tubes.

2. Potter-Elvehjem homogenizer (6) with a loose-fitting pestle.

3. Multiple stirrer. The extractions were performed with a battery of motor-driven spiral glass stirrers mounted above a movable test tube holder. At the end of each extraction the stirrers were washed down with pure petroleum ether.

4. Layer separator. The aqueous alkaline layer of saponifiable matter and the layer of petroleum ether containing the unsaponifiable matter were separated by means of a pipette similar to one previously described (4), but operated by suction.

5. Adsorption columns. The columns of alumina were supported in glass tubing 25 cm. long × 4 mm. inside diameter, tapered at one end, and plugged with glass wool. The alumina was introduced into these tubes by pouring in a slurry made with petroleum ether. The tubes were held in a rubber stopper inserted in a 1 liter suction flask, which was cut off at the bottom and ground to fit a piece of ground plate glass. This arrangement allowed the easy exchange of receptacles.

PROCEDURE

Standard solutions of the recrystallized azo compounds in aldehyde-free 95 per cent ethanol were used

in the determination of the absorption maxima and Evelyn colorimeter constants in 7 *N* HCl. In acid solutions of these dyes there is a distinct gradation of color from the pink-red of the dimethyl compound to the orange of the non-methylated dye. With the Coleman Universal Spectrophotometer, *p*-aminoazobenzene in HCl was found to have an absorption maximum at 500 mμ, while *p*-monomethylaminoazobenzene and *p*-dimethylaminoazobenzene showed maxima at 505 mμ. and 518 mμ. respectively. Evelyn colorimeter filters 500 and 520 were found most suitable for the determination of these compounds. The colorimeter constant, K, in the relation C=KL, where C=concentration in μgm./10 ml. and L=optical density, was determined for each dye and found to be 41.7_{500} and 53.8_{520} for *p*-aminoazobenzene, and 28.4_{520} for each of the other dyes. The two filters gave nearly the same K value for *p*-monomethylaminoazobenzene, since this dye has a very broad absorption curve in acid, but the 500 and 520 filters were best for *p*-aminoazobenzene and *p*-dimethylaminoazobenzene respectively. The value of K_{520} for *p*-aminoazobenzene is given so that it may be used in computing an average constant for the determination of "total azo compounds" when the approximate distribution of the dyes in mixtures is known.

The hydrolysis and extractions were carried out in 1×8 inch test tubes. No more than 4 gm. of fresh tissue were used per tube. The minced tissue was homogenized as finely as possible with 5 ml. of water and the homogenate then transferred quantitatively to a test tube with the aid of 7 ml. of 95 per cent ethanol. Two ml. of 11 *N* aqueous KOH were added, and the whole was mixed well and allowed to stand with occasional shaking for 1½ hours at room temperature. Ten milliliters of petroleum ether were then added and the mixture was rapidly stirred by a motor for 5 minutes. The mixture usually formed a clear layer of petroleum ether on standing or after light centrifugation. When it failed to do so, a few drops of alcohol sufficed to break the emulsions. The upper layer was transferred to another test tube with the aid of the layer separator.

The extraction was repeated two more times and the three extracts were combined. Exactly 10 ml. of the 7 *N* HCl were then added to the combined extracts, and the tube was closed tightly with a rubber stopper and twice shaken vigorously, each time for about 15 seconds. In this manner the azo dyes in the tissue were brought into an acid solution largely free from other unsaponifiable matter. Three extractions removed 95 to 100 per cent of the dyes present providing not more than 100 μgm. of the dyes were present in the original sample. If only one azo dye was known to be present, or the amount of "total azo" compounds was desired, the acid extraction was performed in an Evelyn colorimeter tube and the optical density of the clear acid layer read directly. The volume of the acid layer was then estimated to 0.1 ml. in a graduate having 0.2 ml. divisions, and the colorimeter reading corrected accordingly. Due to the alcohol extracted in the process the final volume was generally found to be 10.2 to 10.4 ml. It was advantageous to read the acid extracts at this point routinely, since this provided a measure of the overall recovery after fractionation on the column.

To fractionate the acid extract of the azo dyes the petroleum ether layer containing the unsaponifiable matter was removed, the tubes were immersed in cold water, and 9 ml. of 11 *N* KOH added slowly so that the temperature of the mixture did not exceed 60° C. The alkaline solution was then extracted with three 10 ml. portions of petroleum ether, as in the case of the tissue hydrolysate. The extracts were collected in a 125 ml. Erlenmeyer flask, a few boiling chips added, and the solvent was removed in vacuo at 50-60° C. The flask was then washed down successively with four 1 ml. portions of petroleum ether and these washings were quantitatively transferred to and adsorbed on a column of alumina 12 cm. long and 4 mm. in diameter. After washing through with 0.5 ml. of petroleum ether, 1 ml. of a 1:1 mixture of petroleum ether and benzene was passed through the column and then followed with pure benzene. During the adsorption from the petroleum ether a band of *p*-dimethylaminoazobenzene often formed below the combined bands of the other azo dyes; as the amount of benzene in the wash solution was increased, three bands formed and traveled down the column. As the bands neared the end of the column, the spaces between the bands varied from 1.5 to 2.0 cm.; complete elution of each band unmixed with the others was accomplished by eluting each band until the band above approached to within a few millimeters of the end of the column. Each band was eluted into separate Evelyn tubes and the tip of the column and walls of the Evelyn tube were washed down with 10 ml. of petroleum ether. Ten milliliters of 7 *N* HCl were added and the colors developed and read. The volume of the acid layer under these conditions was always 10.0 ml., since alcohol was absent.

The only precautions found to be necessary for good recoveries with this procedure were: (a) that undue heat be avoided at all steps, *i.e.*, during neutralization or distillation, and (b) that the azo dyes be permitted to remain in any one solution for no more than 2 to 3 hours. Direct fractionation of the first extract of unsaponifiable matter was not feasible, since the necessary evaporation of the solvent to remove the alcohol

present left residues that did not dissolve in the small volumes of petroleum ether desired for the chromatographic separation. The insoluble particles then clogged the column. If this difficulty could be overcome, direct adsorption would be very convenient, since most non-azo colored compounds in tissue extracts are strongly adsorbed and eluted only very slowly by benzene.

Recovery Experiments

The practical utility of the method was evaluated by means of recovery experiments carried out with the pure dyes, singly or in mixtures, and in the presence or absence of animal tissue at the initial step. Each step of the method was tested with pure solutions containing 5 to 10 μgm. of each dye; 95 to 100 per cent recovery was realized in each case. The presence of tissue up to 4 gm. per tube did not lower the percentage recovery and in many instances favored a more rapid extraction of the dye. The final test of the method was made by adding mixtures containing 5 μgm. of each dye to each of the following rat tissues and excreta: tissue and contents of stomach; first and second halves of small intestine taken separately; cecum and large intestine; liver; kidneys; spleen; heart, lungs, and brain; blood; urine; feces. Each dye was then recovered as described in the procedure. Recoveries of 90 to 95 per cent of the *p*-dimethylaminoazobenzene, 85 to 95 per cent of the *p*-monomethylaminoazobenzene, and 80 to 85 per cent of the *p*-aminoazobenzene were obtained. In the routine analysis of tissues the values for each dye were corrected according to the average recovery found.

Small unavoidable losses in the separate steps probably accounted for the total loss observed. Thus if a recovery of 95 per cent is realized at each of 3 successive steps, the over-all recovery would be only 86 per cent. The greater loss in the case of the *p*-aminoazobenzene was probably due to its lower rate of extractability from aqueous solutions as compared with the other dyes. Large losses, up to 50 per cent of dye, particularly with *p*-aminoazobenzene, were observed when the original tissue saponification was conducted under reflux. Tissue substances apparently were responsible, since each dye was stable to refluxing in pure alcoholic KOH. Smaller, irregular losses of 5 to 15 per cent were noted when the dyes were allowed to stand in the cold with alcoholic KOH and tissue for more than 3 hours, or if the dyes were permitted to remain at any other point in the procedure for more than a few hours. Similar losses were also encountered in the original grinding and saponification when tissue samples greater than 4 gm. per tube were employed. This was particularly true for liver.

In no case during the recovery of each of the azo dyes from mixtures with tissue was more than one band noted on the column at the end of the procedure. Even when partial destruction of the dye occurred on heating or standing, the dye remaining was homogenous. Thus the occurrence of more than one dye in the tissues of animals treated with but one dye (5) can be regarded as real rather than as an artifact resulting from the manipulations.

Errors and Limits of Method

The colorimetric method of estimating the dyes was found to be sufficient to measure as little as 0.5 μgm. of *p*-dimethylaminoazobenzene or *p*-monomethylaminoazobenzene and 1.0 μgm. of *p*-aminoazobenzene to within ±5 per cent. Below these levels the error increased sharply, since the galvanometer readings lay in the range 96 to 100 on a non-linear scale where the blank = 100. Similarly during the chromatographic separation of the three dyes it was difficult to see a band of 0.5 μgm. or less of each dye, even on the small adsorption columns employed. Of course, elutions of apparently blank spaces on the chromatograph are possible if at least one recognizable band is present. Hence the lower limit of the method in both the detection and measurement of these azo dyes in tissue would appear to be of the order of 0.5 μgm.

The method is apparently specific for *p*-aminoazobenzene and its derivatives that contain no acidic group, and it has been used successfully in a study of the demethylation of *p*-dimethylaminoazobenzene in the tissues of the rat (5). Other possible metabolites of the three dyes, *i.e.*, hydroxy derivatives, would not be extracted from the alkaline hydrolysates. Normal tissues do not contain any significant quantity of chromogenic material. Occasionally recoveries at the first step of about 105 per cent have been obtained from digestive tract tissue and contents, and particularly from the cecum and large intestine of rats fed a grain diet. The petroleum ether extracts of these samples contained considerable carotenoid material.

SUMMARY

A quantitative method has been devised for the simultaneous determination of *p*-dimethylaminoazobenzene, *p*-monomethylaminoazobenzene, and *p*-aminoazobenzene in rat tissues. The dyes are extracted from the unsaponifiable matter with 7 *N* HCl, and after neutralization are returned to petroleum ether and passed through adsorption columns of suitably prepared alumina. The color intensity of the eluted fractions is then read in 7 *N* HCl. The method is suitable for the recovery of as little as 1.0 μgm. of each dye from tissue.

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On the Methylation and Demethylation of Certain Carcinogenic Azo Dyes in the Rat*

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While the carcinogenicity of *p*-dimethylaminoazobenzene varies profoundly with the composition of the basal diet fed (3, 4, 9, 16-20, 24), similar variations due to diet have not been observed with other types of carcinogens. Hence the question has been raised whether the dietary effects noted with the azo dye might not be concerned with stages in the metabolism of the compound independent of the carcinogenic process (18, 19). Present information on the metabolism of the dye is not sufficient to supply an answer. The most extensive metabolic study to date is by Stevenson, Dobriner, and Rhoads (22), who fed and injected rats with large amounts of *p*-dimethylaminoazobenzene, and isolated the following substances from the urine: *p*-phenylene diamine, *p*-aminophenol, and the N-acetyl derivatives of these bases. The addition to certain enzyme systems of the diamine corresponding to one-half of the *p*-dimethylaminoazobenzene molecule, unsymmetrical or N,N-dimethyl-*p*-phenylene diamine, results in a strong inhibition of enzyme activity (1, 6, 7, 10, 21). This has led to the suggestion by Kensler, Dexter, and Rhoads (6) that the carcinogenicity of the azo dye depends upon the ability of this hypothetical cleavage product to inhibit certain critical enzymes. It is, of course, entirely possible that some derivative, rather than the parent azo molecule, may be the true carcinogen, but so far the carcinogenicity of N,N-dimethyl-*p*-phenylene diamine, and indeed its very existence in the tissues of rats fed the azo dye, remains to be demonstrated. Kinosita (11) has asserted that this diamine appears in the urine of rats fed the dye, without stating, however, either the method of detection or characterization of the compound. Stevenson and her associates (22) failed to find the diamine [1] in the urine of their rats.

The products isolated by Stevenson and her associates (22) indicate that methyl groups are removed at some time during the metabolic decomposition of *p*-dimethylaminoazobenzene. These groups appear to be physiologically available in minimizing the effects of choline deficiency (5). If all the azo dye were demethylated prior to cleavage, the enzymatically harmful N,N-dimethyl-*p*-phenylene diamine could not be formed. Hence the hypothesis of Kensler, Dexter, and Rhoads (6) requires that demethylation should take place after rather than before reduction of the azo linkage. It is the purpose of the present communication to demonstrate that the rat has the ability to demethylate *p*-dimethylaminoazobenzene prior to cleavage at the azo group.

METHODS

Adult albino rats 150 to 200 gm. in weight were fed diets *ad libitum* containing either 0.06 per cent *p*-dimethylaminoazobenzene, 0.056 per cent *p*-monomethylaminoazobenzene, or 0.053 per cent *p*-aminoazobenzene for periods of 2 to 6 weeks. The latter levels are the molar equivalents of the carcinogenic level of 0.06 per cent of the dimethyl compound. The basal diet was the semisynthetic one that had been found to produce a high percentage of liver tumors in rats at 4 months when 0.06 per cent of *p*-dimethylaminoazobenzene was fed (18). It had the following composition: crude casein 120 gm., salts 40 gm., corn oil 50 gm., rice bran concentrate 20 gm., glucose 770 gm., and riboflavin 0.5 mgm. per kg. One drop of halibut liver oil per rat was fed at the start of the experiment. Food consumptions were determined during the 24 hours previous to analysis. For the analyses of the blood 2 ml. were withdrawn by cardiac puncture under light ether anesthesia. The animal was then killed with ether, the individual organs were removed, washed if necessary to remove excess blood, and analyzed for their contents of each of the three azo dyes by the method developed for this purpose (14). The sections of the gastrointestinal tract and their contents were generally analyzed together. Although it was difficult to separate completely the contents from the tissue, essentially the same distribution of dyes was noted in each with the contents containing most of the dye present.

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[1] In the paper by Stevenson and her associates (22) N,N-dimethyl-*p*-aminoazobenzene and N,N-dimethyl-*p*-phenylene diamine are referred to as the N,N′-compounds.

The criteria for the identity of the compounds found in the liver and blood of rats fed *p*-dimethylaminoazobenzene, *p*-monomethylaminoazobenzene, or *p*-aminoazobenzene were their adsorption characteristics and their absorption spectra. Each compound found *in vivo* was mixed with approximately the same amount of the authentic compound and the mixture chromatographed (14) on an 18 cm. column of alumina. In each case no separation was noted over the entire length of the column. Similarly, the absorption spectra of the compounds obtained from tissue in 7 *N* HCl were indistinguishable from the spectra exhibited by the known compounds.

The presence of demethylated derivatives of *p*-dimethylaminoazobenzene in the tissues of rats fed this dye was regarded as real rather than as an artifact of the determination. Chemical treatments of the dye, such as excess saponification with tissue and overheating, can result in the partial destruction of the compound, but the azo dye recovered is the original *p*-dimethylaminoazobenzene (14). Nor has any evidence been obtained of methylation *in vitro* of the monomethyl dye by intensified treatments similar to those involved in the method of analysis.

RESULTS

Rats fed p-dimethylaminoazobenzene.—Table I presents data indicating that *p*-dimethylaminoazobenzene can be demethylated *in vivo* to form *p*-monomethylaminoazobenzene and *p*-aminoazobenzene. The parts of the gastrointestinal tract below the stomach contained all three forms, with *p*-aminoazobenzene predominating and the monomethyl compound present only in traces. The stomach, as expected, contained the compound fed, and traces of the demethylated derivatives were also present, especially when feces had been consumed. The liver contained all three forms of the dye: an average of 3 μgm. of the dimethyl compound, 1 μgm. of the monomethyl form, and 10 μgm. of *p*-aminoazobenzene. The blood contained much higher concentrations of dye than any of the solid tissues and, contrary to what might be expected, all was present as *p*-aminoazobenzene, 11 μgm. average per ml. of whole blood. The dye was located entirely in the cells; it could not be detected in the plasma of unhemolyzed blood. Many determinations showed that the diets fed did not alter the cell volume from its normal value of nearly 50 per cent (2); hence the dye contained in 2 ml. of blood was also the amount contained in 1 ml. of red blood cells (Table I). It is, of course, hardly conceivable that *p*-dimethylaminoazobenzene could travel from the digestive tract to the liver without at some time passing through the blood. A failure to find the dimethyl or monomethyl compounds in the blood therefore indicates that the concentrations present were too small to be detected by the method of analysis employed. The largest sample analyzed was 5 ml. of cardiac blood, and the smallest detectable amount of these dyes that would survive the analytical manipulations is roughly 0.5 μgm. (14). Hence the maximum amount of the methylated dyes that could have been present in the cardiac blood was of the order of 0.1 μgm. per ml. Portal blood might, however, be expected to contain larger amounts of the methylated azo dyes than cardiac blood, although preliminary experiments (12) indicate that their concentration in portal blood is also less than 0.1 μgm. per ml.

The organs other than the liver contained mainly *p*-aminoazobenzene, and it appeared that those with the most blood also contained the most dye. The spleen, which became dark and enlarged when an azo dye was ingested, contained the largest amount of *p*-aminoazobenzene per unit weight of tissue. Of 4,210 μgm. of *p*-dimethylaminoazobenzene ingested during the previous 24 hours, 348 μgm., or 8 per cent, could be accounted for in the digestive tract and the tissues as the dye itself or as its two demethylated derivatives. For this calculation the assumption was made that the blood constitutes 6.7 per cent of the weight of a 175 gm. rat (2).

Rats fed p-monomethylaminoazobenzene.—The results of the analyses in Table I indicate that this compound not only loses a methyl group in the tissues of the rat but that in the liver, at least, it may become more highly methylated to form *p*-dimethylaminoazobenzene. Indeed, the concentrations of the three dyes in the liver were essentially the same whether *p*-monomethylaminoazobenzene or *p*-dimethylaminoazobenzene was fed; *i.e.,* on the average 3 μgm. of the dimethyl form, 1 μgm. of the monomethyl form, and 9 μgm. of *p*-aminoazobenzene. This similarity in distribution would suggest that the two methylated azo dyes might possess similar carcinogenic potencies, a suggestion that preliminary experiments (15) show to be true. It is of interest that the monomethyl and dimethyl compounds produce tumors in the rat almost exclusively in the liver, which is the only site in which all three dyes are consistently found when either dye is administered.

In all organs other than the liver and in the intestinal tract, the predominating azo dye was *p*-aminoazobenzene, with traces of the monomethyl compound also present. Again the highest concentrations of dye were found in the red blood cells, where only the completely demethylated compound could be detected. Th over-all recovery of the three dyes was 8 per cent (372 μgm.) of the 4,570 μgm. of *p*-monomethylaminoazobenzene consumed during the previous 24 hours.

Rats fed p-aminoazobenzene.—There was no evi-

dence of methylation when this compound was fed to the rat, and the only form of the dye that could be detected in the tissues was *p*-aminoazobenzene itself (Table I). The over-all recovery of this dye in the digestive tract and the tissues was of the same order of magnitude as when the di- or mono-methylaminoazobenzenes were fed, although in most tissues, and also in parts of the digestive tract, the concentrations of *p*-aminoazobenzene present were somewhat higher than after the ingestion of the other compounds. The blood, for example, contained an average of 28 μgm. of the dye per ml. of red cells when *p*-aminoazobenzene was fed, in contrast to averages of 25 and 22 μgm. after the feeding of the mono- and di- methylaminoazobenzenes respectively. The slightly higher levels of *p*-aminoazobenzene in the blood and tissues of rats fed this dye were apparently due to the higher amounts of food, and therefore of dye, ingested *ad libitum* by the rats fed the noncarcinogenic *p*-aminoazobenzene as compared to its more toxic N-methyl derivatives.

Table I: The Distribution of *p*-Dimethylaminoazobenzene and Its Demethylated Azo Derivatives After Ingestion of Each Dye

(Average contents in μgm. per rat; ranges in parentheses; ± = occasional traces < 0.5 μgm.)

Dye fed	No. of rats	Amount of dye consumed in previous day	Dye found	Stomach	Small intestine		Cecum and large intestine	Liver	Kidneys	Spleen	Heart, lungs, brain	Blood, μgm./ml. RBC
					1st half	2nd half						
		4,210	DAB	132 (48-443)	2 (2-3)	±	1 (1-2)	3 (2-4)	±	±	±	–
DAB*	6	(3,300-	MAB	4 (2-7)	±	±	±	1 (1-3)	±	–	–	–
		4,800)	AB	±	9 (2-16)	26 (3-54)	7 (4-10)	10 (5-14)	4 (2-6)	10 (9-12)	10 (5-15)	22 (15-25)
		4,570	DAB	±	±	±	–	3 (2-5)	–	–	–	–
MAB*	6	(3,000-	MAB	160 (80-270)	3 (1-5)	3 (2-4)	2 (1-4)	1 (1-2)	±	±	±	–
		5,100)	AB	±	11 (5-16)	13 (9-16)	4 (2-7)	9 (3-12)	3 (1-5)	9 (6-13)	5 (3-9)	25 (13-29)
		5,930	DAB	–	–	–	–	–	–	–	–	–
AB*	4	(5,100-	MAB	–	–	–	–	–	–	–	–	–
		6,600)	AB	162 (28-367)	14 (11-18)	31 (19-49)	49 (37-62)	14 (11-16)	4 (3-7)	14 (6-21)	18 (8-37)	28 (23-34)

* DAB = *p*-dimethylaminoazobenzene.
MAB = *p*-monomethylaminoazobenzene.
AB = *p*-aminoazobenzene.

Rats injected with p-monomethylaminoazobenzene and p-dimethylaminoazobenzene.—Adult rats were fed the basal diet and injected intraperitoneally with 6.0 mgm. of the dimethyl or 5.6 mgm. of the monomethyl compound in 0.5 ml. of corn oil daily for 1 week or more. Blood samples were obtained by cardiac puncture and the animals killed 18 hours after the last injection. Since the injected dye coated the surface of the peritoneal cavity, it presented a serious source of contamination in the analyses of the various organs, but this error was largely avoided by removing the sections of the digestive tract only after they had been tied off, and by washing these sections and the organs twice with 10 to 15 ml. of acetone before analysis.

The distribution of azo dyes in the tissues of the injected animals (Table II) was very similar to that found in rats that had been fed the dyes (Table I). The dye that was injected and *p*-aminoazobenzene were noted in the gastrointestinal tract and, as before, the latter dye was predominant, more being found in the lower than the upper parts of the tract. When the monomethyl compound was injected, the organs other than the liver contained only *p*-aminoazobenzene. It is likely that most of the small amounts of the dimethyl compound found in the organs of the rats injected with this dye represented contamination rather than actual dye within the organ. Again the livers of the rats injected with either the mono- or the di-methyl compound presented nearly identical distributions and levels of all three dyes; and the over-all picture was very similar to that obtained when the dyes were fed.

The disappearance of the dyes from the body.—The persistence of the dyes in the body was determined by the analysis of rats from which the dye was withheld and to which the basal diet was fed for 1 or 3 days

after a dye-feeding period of 2 to 6 weeks (Table III). The disappearance of each dye from the tract was variable; percentage losses ranged from 50 to 90 per cent after 1 day, and 85 to 98 per cent after 3 days. Smaller losses, of 25 to 38 per cent after 1 day and 56 to 88 per cent after 3 days, were found in the organs other than the liver. These organs contained only dyes found in the urine and feces per rat per day resembled the situation in the digestive tract of these rats at any instant. Rats fed *p*-aminoazobenzene excreted only this dye; approximately 10 μgm. were found per rat per day in the urine and in the feces. Rats fed *p*-monomethylaminoazobenzene excreted nearly 19 μgm. and 10 μgm. of *p*-aminoazobenzene and

TABLE II: THE DISTRIBUTION OF DYES IN THE BODY AFTER INJECTION OF DYE
(Average contents of 2 rats in μgm. per rat; ± = occasional traces of < 0.5 μgm.)

Dye injected	Amount of dye injected in previous 18 hours	Dye found	Stomach	Small intestine 1st half	Small intestine 2nd half	Cecum and large intestine	Liver	Kidneys	Spleen	Heart, lungs, brain	Blood μgm./ml. RBC
		DAB	2	1	3	3	7	3	2	1	—
DAB*	6,000	MAB	1	—	—	—	1	—	—	—	—
		AB*	3	8	25	9	13	3	6	3	29
		DAB	—	—	—	—	2	—	—	—	—
MAB*	5,600	MAB	3	±	—	—	1	—	—	—	—
		AB	8	9	21	6	17	5	11	11	18

* See footnote to Table I.

TABLE III: RATES OF DISAPPEARANCE OF DYES FROM BODY AFTER FEEDING OF DYE IS STOPPED
(Average of 2 rats expressed as percentage loss from normal average; ± = trace < 0.5 μgm.)

Dye fed	Days off dye	Dye found	Stomach	Small intestine 1st half	Small intestine 2nd half	Cecum and large intestine	Liver	Kidneys	Spleen	Heart, lungs, brain	Blood
		DAB	81	—	—	—	30	—	—	—	—
	1	MAB	—2	—	—	—	0	—	—	—	—
		AB	—	86	89	81	49	27	—26	29	22
DAB*		DAB	92	50	±	>50	67	—	—	—	—
	3	MAB	>87	±	±	±	>50	—	—	—	—
		AB	—	89	92	86	79	67	61	lost	73
		DAB	±	—	—	—	67	—	—	—	—
	1	MAB	84	63	60	12	>50	—	—	—	—
		AB	±	70	72	40	50	37	—2	38	33
MAB*		DAB	—	—	—	—	>50	—	—	—	—
	3	MAB	98	±	±	±	—	—	—	—	—
		AB	—	89	85	—7	55	57	78	74	78
		DAB	—	—	—	—	—	—	—	—	—
	1	MAB	—	—	—	—	—	—	—	—	—
		AB	97	89	88	78	61	25	—36	88	31
AB*		DAB	—	—	—	—	—	—	—	—	—
	3	MAB	—	—	—	—	—	—	—	—	—
		AB	99	90	95	92	84	65	56	88	70

* See footnote to Table I.

p-aminoazobenzene; the occasional negative losses indicate the variability of the blood contents of these organs as removed from the body. The liver suffered a loss of 30 to 67 per cent of its dye content after 1 day. By 3 days the loss in this organ reached 50 to 84 per cent. The blood samples were more uniform in their behavior: A loss of 22 to 33 per cent of the original dye content was noted after 1 day, and by 3 days the loss had reached 70 to 78 per cent.

Excretion of dyes in urine and feces.—Preliminary data indicated that the distribution and amount of the monomethyl compound respectively in the urine, and 1 to 3 μgm. of these dyes in the feces. No dimethyl compound was detected in the excreta of these rats. When *p*-dimethylaminoazobenzene was fed, the urine contained 11 μgm. and 6 μgm. of *p*-aminoazobenzene and the dimethyl compound respectively, and 1 to 4 μgm. of these dyes were found in the feces; only traces of the monomethyl compound were discovered. When the monomethyl and dimethyl compounds were injected, the pigments were excreted in nearly the same amounts as when the dyes were fed. However,

only *p*-aminoazobenzene was found in the feces of the injected animals.

DISCUSSION

The chief conclusions drawn from the present experiments are: (a) that *p*-dimethylaminoazobenzene can be reversibly demethylated in the rat to form *p*-monomethylaminoazobenzene, and (b) that the further demethylation of the monomethyl compound to *p*-aminoazobenzene is not reversible to the extent that the methylated derivatives could be detected by the method employed. Several of the possible metabolic pathways from these compounds to those found in the urine by Stevenson and her associates (22) are shown in Fig. 1. It is apparent from this scheme that much work will be needed before the reactions essential to the process of carcinogenesis by the azo dyes can be disentangled from unrelated reactions.

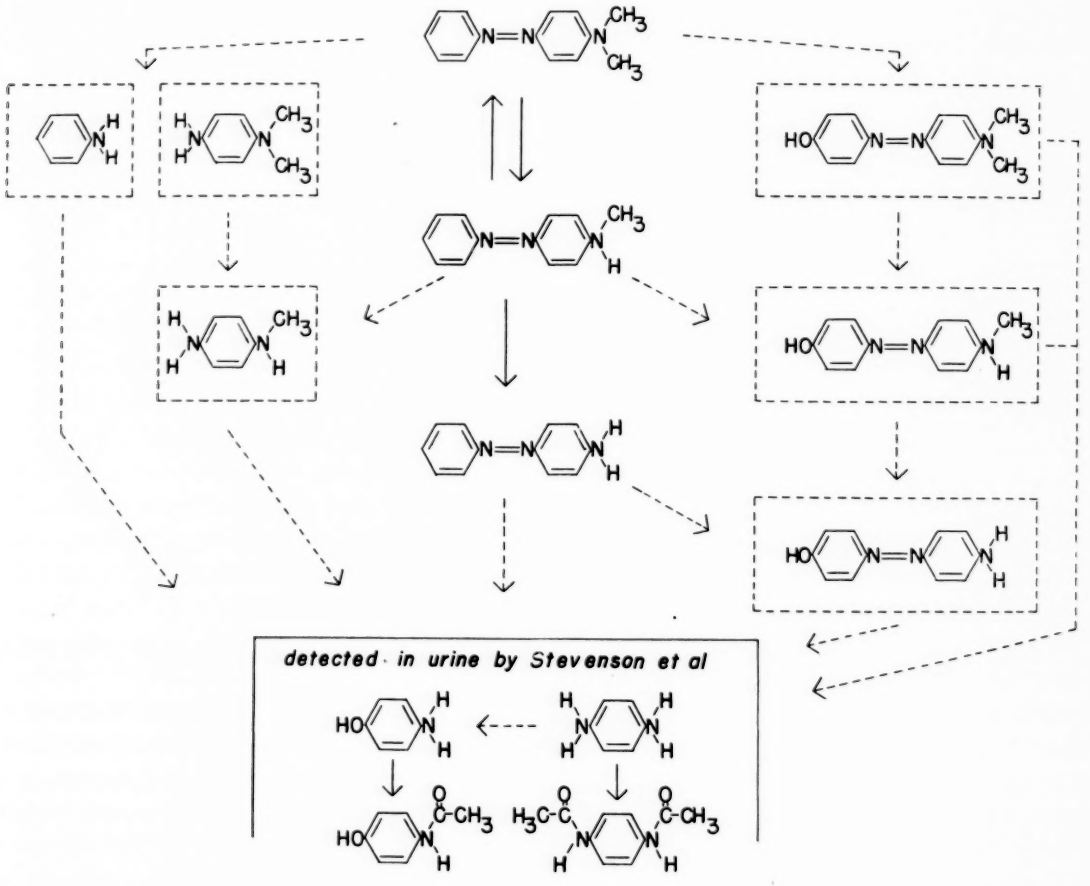

FIG. 1.—Present status of the metabolism of *p*-dimethylaminoazobenzene by the rat. (The derivatives within dotted lines and the reactions indicated by dotted arrows are hypothetical.)

The presence of compounds other than those administered is probably due to reactions within the tissues themselves, since a similar distribution of the three dyes was noted whether the original N-methyl dyes were fed or injected intraperitoneally. This finding tends to eliminate the microorganisms of the digestive tract as agents primarily responsible for the demethylations, in spite of the relatively large amounts of *p*-aminoazobenzene found in the lower parts of the tract. It is possible that the demethylation reactions take place in the liver; and that the presence of the reaction product, *p*-aminoazobenzene, in the digestive tract is the result of excretion through the bile.

As indicated previously, it is extremely important whether demethylation takes place before or after the azo linkage is cleaved. The split-product theory of carcinogenesis by azo dyes (6) can be valid only if some cleavage precedes demethylation; it is now evident that much demethylation precedes cleavage. But whether all the N-methyl dye is demethylated before cleavage cannot be established from the present experiments. The rats had all been fed the usual carcinogenic amounts of the dyes for periods long enough to establish an equilibrium such as exists when tumors are being produced. Under these conditions the dyes recovered accounted for about 8 per cent of that consumed during the previous 24 hours. But the latter time interval is, after all, an arbitrary unit; it probably

has no physiological significance in these experiments. Much further information on the excretion and on the destruction of the various azo dyes in the tissues and in the digestive tract will be necessary before even a preliminary metabolic balance sheet can be drawn. Nevertheless, if it is assumed that the amount of *p*-aminoazobenzene in the blood and tissues is an index of the amount of this substance being formed from the other azo dyes, it can be argued from the similarity of the amounts of *p*-aminoazobenzene in the tissues of rats fed any one of the three dyes (Table I) that virtually all of the N-methyl dyes in the body passes through the *p*-aminoazobenzene stage. Furthermore, the rapid disappearance of the dyes from the body (Table III) would appear to support this argument.

An original weakness in the split-product hypothesis of carcinogenesis was that the critical N,N-dimethyl-*p*-phenylene diamine had never been demonstrated in the tissues or excreta of the rat. This weakness is now aggravated by the demonstration that *p*-dimethylaminoazobenzene yields products from which the critical split product could not be formed. Other difficulties are as follows: The products of oxidation of N,N-dimethyl-*p*-phenylene diamine are very toxic *in vitro* towards systems containing nicotinic acid or thiamine as a component of the coenzyme (6, 10), yet these vitamins fail to exert an appreciable effect on the carcinogenicity of the parent azo dye (19). On the other hand, riboflavin delays considerably the formation of liver tumors by the dye (9, 19) and the dye hastens the excretion of the vitamin (8), yet there is no evidence that the diamine or its oxidation products are particularly toxic toward enzyme systems containing riboflavin. In fact, *d*-amino acid oxidase was found to be resistant to the diamine (7). Furthermore, *p*-phenylene diamine, the probable metabolic cleavage product of the noncarcinogenic compound, *p*-aminoazobenzene, is also toxic to enzyme systems and liver cells (13), though to a lesser degree than the dimethyl diamine, the compound on which speculation has been based. Finally, N,N-dimethyl-*p*-phenylene diamine has been fed to rats for long periods of time without the production of liver tumors (11, 15, 23).

SUMMARY

1. The tissues of rats fed *p*-dimethylaminoazobenzene contained not only the dye itself, but its demethylated derivatives *p*-monomethylaminoazobenzene and *p*-aminoazobenzene. The amounts of these three dyes found in the body accounted for approximately 8 per cent of the dimethyl compound ingested during the previous 24 hours. Most of the dye was located in the red blood cells in the form of the completely demethylated derivative, *p*-aminoazobenzene.

2. When *p*-monomethylaminoazobenzene was fed to rats, the liver contained both *p*-aminoazobenzene and *p*-dimethylaminoazobenzene in addition to the dye fed; the blood contained only *p*-aminoazobenzene. The distribution of the three dyes was quantitatively the same whether the monomethyl or dimethyl derivative was fed. The presence of *p*-dimethylaminoazobenzene in the livers of rats fed the monomethyl compound indicates that the rat is capable of methylating the latter substance.

3. When either the monomethyl or dimethyl compound was fed, or injected intraperitoneally, the contents of the intestinal tract contained both the dye administered and *p*-aminoazobenzene, with the latter dye predominant. The distribution of the three dyes in the tissues was essentially the same whether the monomethyl or dimethyl compound was fed, or injected intraperitoneally. When feeding of the dye was stopped, the concentration of all three forms in the tissues decreased rapidly.

4. When *p*-aminoazobenzene was fed to rats, the only form of dye found in the tissues was *p*-aminoazobenzene itself.

5. These findings demonstrate that *p*-dimethylaminoazobenzene is demethylated *in vivo* prior to reduction at the azo linkage. The high levels of *p*-aminoazobenzene in the body and the rapid rate of disappearance of the three dyes therefrom indicate that demethylation prior to cleavage constitutes a major pathway in the metabolism of the methylated dyes. It is possible that the metabolism of *p*-dimethylaminoazobenzene follows this pathway to such an extent as to preclude the formation of N,N-dimethyl-*p*-phenylene diamine, the alleged carcinogen according to one current theory.

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The Effects of Roentgen Radiation on the Thymonucleic Acid Content of Transplantable Mammary Carcinomas*

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Roentgen radiation has the ability to produce histologic changes in tissues and the death of cells. Many kinds of tumor cells are more sensitive to its lethal effects than the corresponding normal cell types from which they arise, but the intracellular mechanism by which the rays cause the death of normal and neoplastic cells is not definitely known. It has been suggested by Henshaw (9) that they alter the molecular structure of vital nuclear material, perhaps of the proteins of the chromosomes, in a manner that is incompatible with the further life of the cell. Because of the importance of nucleoproteins in the vital function of normal and neoplastic cells it is desirable to understand how they are altered by roentgen energy. To determine the effects of roentgen rays on the thymonucleic acid content of transplantable mammary carcinomas in rats and in mice, two series of experiments were undertaken.

MATERIALS AND METHODS

These investigations were conducted as two similar experiments employing the use of rats in the first and of mice in the second experiment.

Rats.—Eight young rats of the August strain were obtained through the courtesy of Dr. Wm. H. Woglom. Two of these rats bore transplants of mammary carcinoma R 2426, which had been passed through many generations of rats of this strain in the laboratories of the Department of Cancer Research at Columbia University. This tumor has been used in numerous experimental problems by Eisen and Woglom (4-8).

Grafts from one of the tumors were transplanted beneath the skin of the flanks and abdomen of the rats, 3 or 4 on each side of each animal. After 5 weeks, about three-fourths of the transplants were recognizable as subcutaneous tumor nodules with an average diameter of 1 cm.

At this time the carcinomas on one side of each rat were irradiated, while the control neoplasms on the opposite side of the animal were protected by lead shielding. The roentgen radiation was administered while the rats were tied securely on their backs to small boards. The radiation was given in 3 different dosages. The tumors of 2 animals were given a single treatment of 4,000 r at a dosage rate of 300 r per minute and a skin-target distance of 15 cm. The 2 other pairs of rats received repeated treatments of 500 r every other day until totals of 2,000 and 4,000 r had been administered. For these treatments the skin-target distance was 50 cm. and the dosage rate was 60 r per minute. A 200 kv. x-ray machine was used with a filter of 0.25 mm. of copper for all the rats and mice. At intervals of 90 minutes, 20 hours, 1 week, and 2 weeks following the last treatment, tumors were removed for microscopic examination. The interval of 90 minutes is sufficient to allow cells in the process of division at the time of radiation to complete their mitoses. For each irradiated tumor, a control tumor was removed at the same time from a corresponding region on the other side of the body. These control tumors were situated a sufficient distance from the radiated ones so that backscattering of the radiant energy was assumed to have no effect on them. The rats were anesthetized with ether for the removal of the tumors.

The tumors were fixed in equal parts of a saturated aqueous solution of mercuric chloride and 95 per cent ethyl alcohol for 18 to 20 hours. They were then given several changes of 70 per cent alcohol and, under conditions standardized by the use of an autotechnicon, dehydrated through a graded series of solutions of ethyl alcohol, cleared in chloroform, and infiltrated with parowax.

In a manner similar to that employed in the other photometric studies on thymonucleic acid (18, 21),

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serial sections were cut at 10 microns, mounted on slides of uniform measured thickness at a temperature of 40±0.5° C., and stained with hematoxylin and eosin and with the Feulgen reaction. The 24 sections of radiated and control tumors were arranged on 6 slides so that variations in staining might be reduced. In addition to the 2 adjacent serial sections of each tumor stained by the Feulgen reaction, a third, adjacent, section was prepared as an unstained control.

Mice.—The second part of the experiment involved observations on the effects of roentgen radiation on a transplantable mammary carcinoma in the C57 strain of mice. Young C57 mice, as well as the transplantable tumor, were obtained from the Roscoe B. Jackson Memorial Laboratory. This rapidly growing carcinoma had been transplanted through 45 generations (11). In a total of 14 mice, 2 transplants were made into the subcutaneous tissues on each side of the ventral abdominal wall. After 3 weeks the transplants had grown to an average diameter of 1 cm., and the tumors on one side of the body were then treated with x-ray in a manner similar to that used for the rats. The carcinomas were exposed through holes in lead shielding that protected the remainder of the body surface of the mouse.

The tumors of 3 mice received a single treatment of 4,000 r, which was given with a skin-target distance of 15 cm. at a dosage rate of 300 r per minute. Four other mice were given treatments of 250 r on each of 4 alternate days, making a total of 1,000 units. The carcinomas of 3 mice were given 2,500 r, with 250 units on each of 4 alternate days and 500 units on the next 3 subsequent alternate days. These treatments were given with a skin-target distance of 50 cm., and the dosage rate was 60 r per minute. Three additional mice died as a result of accidents or treatment before any tumors were removed. Tumors were extirpated at intervals of 90 minutes, 20 hours, 40 hours, 3 days, 5 days, and 6 days following the last treatment. As in the previous experiment, the animals were sacrificed whenever possible before they became moribund and the remaining tumors were removed. The tissues were fixed and sections prepared and stained with hematoxylin and eosin and by the Feulgen reaction in the same manner as for the rat carcinomas. The 31 sections of the radiated and control mouse tumors were arranged on 5 different slides.

Apparatus.—Microphotometric apparatus has been constructed that is suited for measurement of the absorption of light by pigments in tissues. It consists of a light source and filters, a microscope, a photocell, and amplification and recording apparatus (18), and when a stain is employed that is specific for one tissue constituent the relative amounts of this component in various areas of the same or different sections of tissue may be determined. Although such an apparatus has many possible uses in histochemical research (19), it has been employed only in studies on thymonucleic acid (18, 21). In this work the assumption is made, which for practical purposes seems justified (18-21), that the amount of complementary monochromatic light absorbed by the pigment in tissues treated by the Feulgen reaction is directly proportional to the amount of thymonucleic acid present.

The sections of rat and mouse tumors stained by the Feulgen reaction were measured with the photometric apparatus in the same manner, with methods already described (18). A diaphragm in the ocular of the photometric apparatus limited the stage field to an area of 32 by 42 microns. The absorption of light was measured on 50 adjacent areas on each of the sections. The nuclei in each field were counted so that computations could be made of the mean amount of absorption of light per cell. In making the measurements, areas were avoided that showed degeneration or death of cells, polymorphonuclear leukocytes, lymphocytes or macrophages, hemorrhage or artefacts.

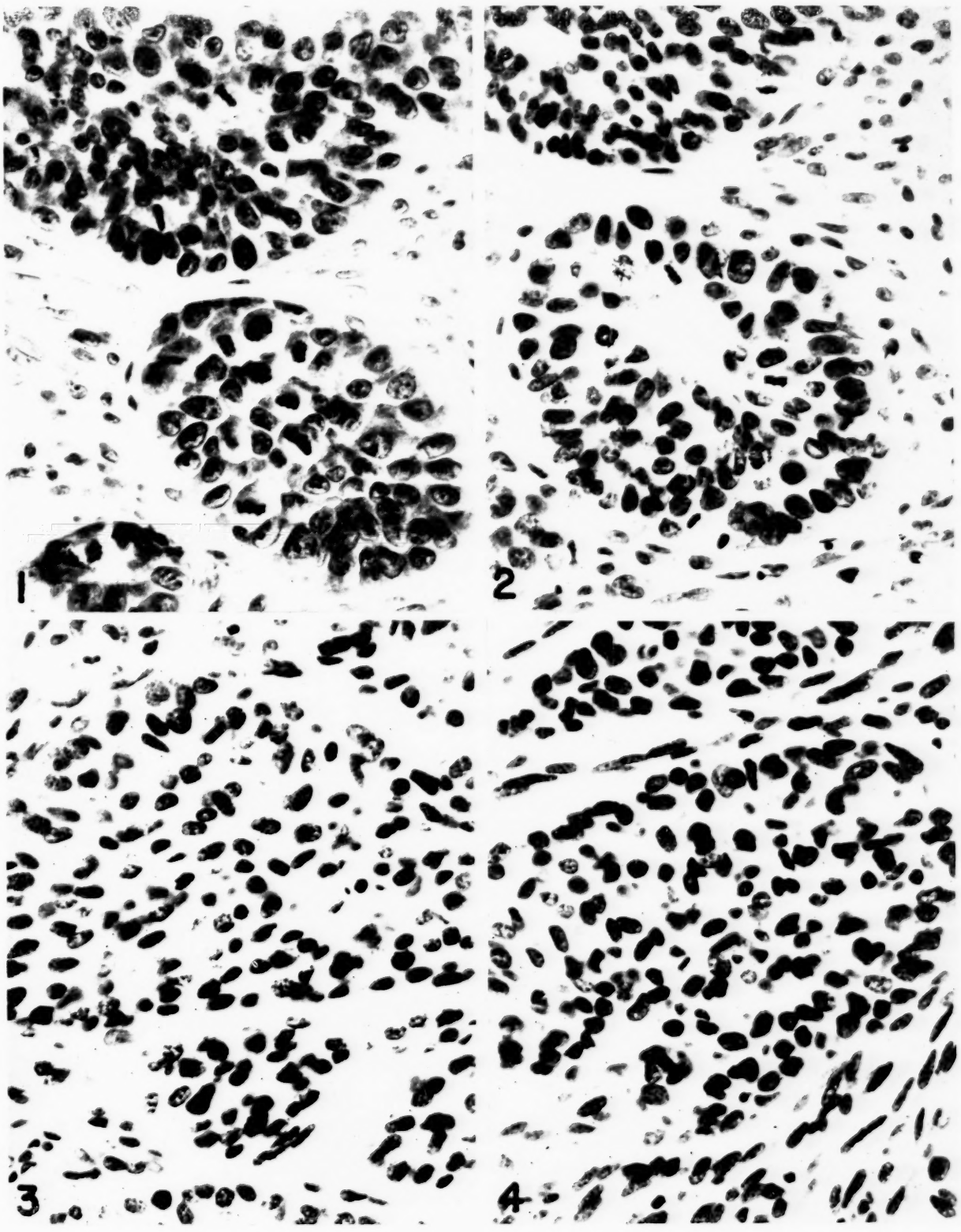
RESULTS

Rats.—There was no grossly recognizable regression and only equivocal retardation of the growth of any of the rat carcinomas following radiation. Although 28 were removed from 6 rats, 4 carcinomas were considered unsuitable for analysis. One animal bearing 3 carcinomas moved during the radiation, hence none of its tumors, which had received a questionable amount of radiation, were measured.

The 24 tumors that were measured showed considerable variation in their histologic appearance. The tumor cells tended to be arranged in acini and in solid cords (Figs. 1 and 2). The amount of fibrous tissue and necrosis was not always readily correlated with the radiation of the tumors. The larger carcinomas were more necrotic than the smaller, but by cutting sections from the periphery instead of from the center the amount of necrosis in the sections was reduced.

Even though the identity of the sections was unknown the radiated tumors could be recognized histologically in every instance when compared with the corresponding controls. The differential characteristics of the radiated tumors were a decreased number of mitotic figures and slightly smaller and more hyperchromatic nuclei. For the tumors with a longer interval between radiation and biopsy there was a somewhat variable tendency for an increased number of dead cells and more fibrous tissue in the viable parts of the neoplasm.

The results of the measurements of relative amounts



FIGS. 1 and 2.—Adjacent areas of a nonirradiated transplanted rat mammary carcinoma (No. 5Ca) showing numerous mitotic figures. Fig. 1, stained with hematoxylin and eosin, shows the cytoplasm, while Fig. 2, stained by the Feulgen reaction, does not. Mag. × 460.

FIGS. 3 and 4.—Areas of transplantable mammary carcinoma in C57 mice, showing the apparent similarity between the thymonucleic acid content of comparable control tumor (Fig. 3, section 1 Ca) and tumor receiving 4,000 r of x-radiation (Fig. 4, section 1Rb). The mean computation of 50 areas of each section showed a statistically significant decrease in the radiated tumor in the thymonucleic acid content per area and per cell. Ten micron section. Feulgen stain. Mag. × 460.

of thymonucleic acid in these radiated transplantable mammary carcinomas and their controls are shown in Table I. The results are expressed in terms of

Table I: Relative Thymonucleic Acid Content Per Unit Volume of Tissue and Per Cell, as Measured by Light Absorption on Radiated and Nonradiated Mammary Carcinomas in Rats

Rat tumor no.*	Treatment, r	Time between biopsy and treatment	Absorption per area, %	Coeff. of variation of area, %	Cells per area, mean no.	Absorption per cell, mean %
1Ca-1	4,000	90 min.	28.4	11.6	29.9	.94
1Ca-2	4,000	90 "	26.1	12.0	25.9	1.01
1Rb-1	4,000	90 "	25.4	8.8	30.1	.84
1Rb-2	4,000	90 "	23.6	10.1	27.8	.85
1Cc-1	4,000	20 hrs.	27.0	11.4	30.0	.90
1Cc-2	4,000	20 "	28.4	11.0	28.7	.99
1Rd-1	4,000	20 "	21.8	21.2	25.1	.87
1Rd-2	4,000	20 "	25.5	10.6	25.0	1.02
2Ca-1	2,000	90 min.	23.7	14.0	27.5	.86
2Ca-2	2,000	90 "	29.7	11.4	27.1	1.10
2Rb-1	2,000	90 "	25.6	10.0	27.3	.94
2Rb-2	2,000	90 "	31.2	8.6	25.7	1.21
3Ca-1	2,000	90 "	24.8	10.2	30.9	.80
3Ca-2	2,000	90 "	26.0	8.5	27.9	.93
3Rb-1	2,000	90 "	24.6	7.5	26.1	.94
3Rb-2	2,000	90 "	24.2	5.7	26.1	.93
2Cc-1	2,000	20 hrs.	20.9	4.3	27.8	.75
2Cc-2	2,000	20 "	25.8	14.4	24.5	1.05
2Rd-1	2,000	20 "	25.9	11.9	31.3	.83
2Rd-2	2,000	20 "	27.6	8.8	26.3	1.04
2Ce-1	2,000	7 days	27.0	10.7	27.3	.99
2Ce-2	2,000	7 "	29.4	10.0	28.0	1.05
2Rf-1	2,000	7 "	23.7	7.5	26.5	.89
2Rf-2	2,000	7 "	29.2	10.1	26.0	1.12
3Cc-1	2,000	7 "	30.4	8.2	31.6	.96
3Cc-2	2,000	7 "	27.2	17.5	29.1	.93
3Rd-1	2,000	7 "	25.0	7.0	31.2	.80
3Rd-2	2,000	7 "	23.1	9.6	25.4	.91
3Ce-1	2,000	14 "	23.8	20.5	28.5	.83
3Ce-2	2,000	14 "	25.3	9.2	25.3	1.00
3Rf-1	2,000	14 "	21.7	9.7	27.3	.79
3Rf-2	2,000	14 "	25.6	10.2	25.7	1.00
4Ca-1	4,000	90 min.	24.7	8.8	30.2	.82
4Ca-2	4,000	90 "	25.5	8.9	26.7	.96
4Rb-1	4,000	90 "	20.6	6.8	25.8	.80
4Rb-2	4,000	90 "	23.2	19.8	23.1	1.00
5Ca-1	4,000	90 "	24.6	8.3	29.6	.83
5Ca-2	4,000	90 "	27.9	6.8	26.6	1.05
5Rb-1	4,000	90 "	21.5	11.5	25.9	.83
5Rb-2	4,000	90 "	26.0	9.6	25.4	1.02
5Cc-1	4,000	20 hrs.	23.6	10.1	29.1	.81
5Cc-2	4,000	20 "	25.5	10.1	25.1	1.02
5Rd-1	4,000	20 "	21.8	9.4	25.2	.86
5Rd-2	4,000	20 "	22.4	10.5	23.1	.97
5Ce-1	4,000	6 days	26.7	16.1	28.4	.94
5Ce-2	4,000	6 "	29.8	10.0	29.8	1.00
5Rf-1	4,000	6 "	21.5	10.0	31.0	.69
5Rf-2	4,000	6 "	24.5	10.6	27.4	.89

* See text for explanation of numbers.

per cent absorption of light per area and per cell. For the data listed under "rat tumor number" in Tables I and II the digits 1 to 5 indicate the number of the rat; C and R represent control and radiated tumors; a, b, c, d, e, and f stand for the different tumors removed from one rat; and 1 and 2 indicate the first and second section cut from each tumor.

The mean absorption per area and per cell for both sections of each rat tumor are given in Table II. Also the ratios for the mean absorptions per area and per cell of the radiated to the control tumors are calculated. The ratios of the absorption per area of the 6 tumors radiated with 4,000 r show that the

thymonucleic acid content per area decreased in each instance. These radiated tumors contained an average of 87.3 per cent as much thymonucleic acid per unit volume of tissue as the controls.

The ratios of the amount of absorption per cell were more variable. The thymonucleic acid content of the radiated cells decreased in 3 instances, remained unchanged in 2, and increased insignificantly in 1 case. The 6 tumors radiated with 4,000 r contained an average of 94.6 per cent as much thymonucleic acid per cell as the nonirradiated control tumors. For the rat tumors that received only 2,000 r the results were inconclusive.

The mean absorption per area for all control and radiated tumors was 26.4 and 24.4 per cent respectively. Thus the radiated tumors contained 92.4 per cent as much thymonucleic acid as the controls. In all the rat tumors, 68,822 cells were measured. There was an average of 26.3 radiated cells per area and of 28.3 nonradiated cells per area. The mean absorption per cell for all control tumors was 0.94 per cent and for all radiated tumors 0.92 per cent.

The data for the probability, P, that an equal or greater positive deviation might be obtained by chance is given in Table II. The figures are averages for comparison of the measurements on 50 areas on 2 similar sets of control and radiated slides. The minus signs before the values for P of tumors 2a and b, 2c and d, and 3a and b indicate that the radiated tumors in these instances contained increased amounts of thymonucleic acid rather than decreased amounts. A value of 0.01 for P means that there is one chance in a hundred of getting as great a difference between the two means by accident. The mean figure for P for the measurements of absorption per area of 12 radiated and control tumors is 0.0003; for the 6 control and radiated tumors receiving 4,000 r is 9.9×10^{-10}; and for the 6 tumors receiving 2,000 r is 0.2061. Following irradiation the amount of thymonucleic acid per cell was increased a statistically significant amount in 2 tumors receiving 2,000 r, and significantly decreased in 3 tumors receiving 4,000 r.

TABLE II: RATIOS FOR RELATIVE ABSORPTION DUE TO THYMONUCLEIC ACID PER UNIT VOLUME AND PER CELL OF RADIATED TO NONRADIATED MAMMARY CARCINOMAS IN RATS

		Absorption per area			Absorption per cell		
Rat tumor no.*	Treatment, r	Absorption, mean %	Ratio of radiated / control	P	Absorption, mean %	Ratio of radiated / control	P
1Ca	4,000	27.2	.90	0.0000	.97	.87	0.0000
1Rb	4,000	24.5			.84		
1Cc	4,000	27.7	.85	0.0000	.94	1.00	0.4761
1Rd	4,000	23.6			.94		
2Ca	2,000	26.7	1.06	— 0.0001	.98	1.10	— 0.0000
2Rb	2,000	28.4			1.08		
3Ca	2,000	25.4	.96	0.0188	.86	1.09	— 0.0019
3Rb	2,000	24.4			.94		
2Cc	2,000	23.4	1.15	— 0.0000	.90	1.05	— 0.0322
2Rd	2,000	26.8			.93		
2Ce	2,000	28.2	.94	0.0003	1.02	.98	0.2743
2Rf	2,000	27.4			1.00		
3Cc	2,000	28.8	.84	0.0000	.94	.90	0.0000
3Rd	2,000	24.0			.86		
3Ce	2,000	24.6	.96	0.1587	.92	.98	0.2033
3Rf	2,000	23.6			.90		
4Ca	4,000	25.2	.87	0.0000	.89	1.01	0.4207
4Rb	4,000	21.9			.90		
5Ca	4,000	26.3	.90	0.0000	.94	.98	0.2236
5Rb	4,000	23.8			.92		
5Cc	4,000	24.6	.90	0.0000	.92	1.00	0.2912
5Rd	4,000	22.1			.92		
5Ce	4,000	28.2	.81	0.0000	.92	.81	0.0000
5Rf	4,000	22.5			.79		

* See text for explanation of numbers.

Mice.—Following radiation, some of the transplantable mammary carcinomas in the mice showed a retardation of growth, a slight regression in size, or an increased softening. Such grossly recognizable results

of radiation, however, were usually equivocal and often absent. Microscopically the tumors consisted of solid masses of neoplastic cells separated by variable amounts of fibrous stroma. The amount of necrosis was more readily correlated with the size of the tumor than with the effects of radiation. It was difficult to distinguish the radiated tumors from the controls, although the relative proportions of mitotic figures, pyknotic nuclei, necrosis, and fibrosis were useful criteria. The similarity between a comparable radiated and control tumor is illustrated in Figs. 3 and 4. However, as shown by the photometric measurements, the mean amount of thymonucleic acid in 50 areas was significantly reduced in the radiated tumor (Fig. 4).

Of the 3 mice that were given a single treatment of 4,000 r, 2 died on the fourth day and the third was killed in a moribund condition on the fifth day after the treatment.

A total of 37 tumors was removed from 11 mice. The 2 tumors of one mouse were discarded because it moved during treatment and exposed all the tumors to some radiation. One pair of tumors from mouse 5 and from mouse 8 was discarded because the radiated carcinoma of each pair showed unusually extensive necrosis, which would make it difficult to obtain readings.

The results of the measurements of light absorption of the transplantable mammary carcinomas in mice are shown in Table III. For the figures listed under

TABLE III: RELATIVE THYMONUCLEIC ACID CONTENT PER UNIT VOLUME OF TISSUE AND PER CELL, AS MEASURED BY LIGHT ABSORPTION ON RADIATED AND NONRADIATED MAMMARY CARCINOMAS IN MICE

			Measurements per area					Measurements per cell		
Mouse tumor no.*	Treatment, r	Time between biopsy and treatment	Cells, mean no.	Absorption, mean %	Mean coeff. of variation, %	Ratio of radiated / control absorption	P	Absorption, mean %	Ratio of radiated / control absorption	P
1Ca	4,000	90 min.	18.8	30.4	10.3	.93	0.0000	1.61	.94	0.0028
1Rb	4,000	90 "	18.1	27.4	9.4			1.52		
1Cc	4,000	20 hrs.	19.1	31.6	9.4	.92	0.0049	1.64	.93	0.0465
1Rd	4,000	20 "	19.0	29.1	11.4			1.53		
2Ca	4,000	20 "	19.2	30.4	7.8	.98	0.1151	1.58	1.02	— 0.1492
2Rb	4,000	20 "	18.5	29.8	8.2			1.62		
2Cc	4,000	40 "	18.8	31.1	8.1	.97	0.0571	1.67	.97	0.0735
2Rd	4,000	40 "	18.9	30.6	10.5			1.62		
3Ca	4,000	5 days	18.4	30.5	10.1	.95	0.0212	1.65	.97	— 0.1867
3Rb	4,000	5 "	18.0	29.1	10.6			1.60		
3Rc	4,000	5 "	17.4	29.1	10.0	.96	0.0031	1.66	1.01	0.1587
4Ca	1,000	90 min.	19.2	29.0	8.9	.96	0.0059	1.50	1.00	0.2877
4Rb	1,000	90 "	18.5	27.7	8.2			1.48		
5Ca	1,000	24 hrs.	18.8	31.4	7.6	.88	0.0000	1.67	.92	0.0009
5Rb	1,000	24 "	18.2	27.8	10.4			1.52		
6Ca	1,000	3 days	18.4	30.6	10.2	.96	0.0228	1.65	.98	0.2236
6Rb	1,000	3 "	18.2	29.3	9.2			1.61		
6Cc	1,000	3 "	18.8	29.4	9.6	.97	0.0778	1.55	1.00	0.4920
6Rd	1,000	3 "	18.4	28.5	10.8			1.56		
7Ca	1,000	6 "	19.2	28.5	10.3	.88	0.0000	1.48	.93	0.0008
7Rb	1,000	6 "	18.2	25.2	8.5			1.38		
7Cc	1,000	6 "	19.5	27.1	9.0	.96	0.0594	1.41	1.04	— 0.2005
7Rd	1,000	6 "	18.0	26.1	9.0			1.45		
8Ca	2,500	90 min.	17.9	27.8	10.0	1.00	0.4681	1.55	.95	— 0.4751
8Rb	2,500	90 "	19.0	27.7	9.0			1.45		
9Ca	2,500	24 hrs.	18.8	30.5	9.6	.93	0.0000	1.61	.91	0.0002
9Rb	2,500	24 "	19.2	28.3	9.2			1.47		
9Cc	2,500	24 "	18.0	28.7	8.8	.95	0.0055	1.59	.98	0.1949
9Rd	2,500	24 "	17.4	27.3	10.4			1.57		
10Ca	2,500	2 days	19.3	29.2	9.5	.93	0.0008	1.50	.98	0.2877
10Rb	2,500	2 "	18.4	27.1	12.7			1.47		

* See text for explanation of numbers.

"mouse number" the numbers 1 to 10 designate the mouse; C and R represent control and radiated tumors; and a, b, c, and d indicate different tumors removed from a particular mouse. To conserve space, only the mean values for each set of 50 measurements on two sections are given. A total of 57,519 cells was measured in the mouse carcinomas. The radiated carcinomas had an average of 18.8 cells per field, as compared with 18.3 per field for the control tumors. The ratios for the radiated to control tumors for the absorption per area and per cell are also given in Table III. All the 16 tumors except 1 showed a decrease in the amount of thymonucleic acid per area following radiation. The mean absorption per area of the control and radiated carcinomas was 29.7 and 28.1 per cent respectively. The radiated tumors contained an average of 94.7 per cent as much thymonucleic acid as the nonradiated. There was more variation, however, in the ratio of the amount of thymonucleic acid per cell of the radiated to the control tumors. The mean absorption per cell for the radiated and control carcinomas was 1.53 and 1.58 per cent respectively. The thymonucleic acid in the radiated tumors was increased in 3 tumors, unchanged in 2, and decreased in 11.

The values for the probability, P, that an equal or greater deviation might be obtained by chance are shown in Table III. For 9 of the 16 sets of measurements the absorption per area was decreased a statistically significant amount. The mean P of the differences in absorption per area for the 4 sets of tumors receiving 4,000 r was 0.0049, for the 6 sets of tumors receiving 1,000 r 0.0006, and for the 4 tumors receiving 2,500 r 0.0078. In 4 instances the amount of thymonucleic acid per cell was significantly decreased following irradiation and in no mouse tumor was it significantly increased.

DISCUSSION

Both mammary carcinomas used for determining the effects of radiation on thymonucleic acid in tumor cells were moderately radioresistant. Although the carcinoma in the C57 mice grew much more rapidly than the rat carcinoma, it was not correspondingly more radiosensitive. Several investigators, including Cramer (1, 2), have found that spontaneous mammary carcinomas in mice and rats vary greatly in their radiosensitivity, while transplanted tumors are relatively constant in their reaction. Samssonow (17), using a 180 kv. x-ray apparatus with filtration through 8 mm. of aluminum at a distance of 30 cm., found that between 9,660 and 13,800 r was necessary to produce regression in 25 spontaneous mammary carcinomas in mice.

Numerous workers have also tested the effects of radiation upon tumors *in vitro*. The tumors were removed from the host, radiated, transplanted into animals of the same strain, and the percentage of takes at different dosages was determined. Packard (15), in summarizing some of his observations, as well as those by Wood (22), found that rat sarcoma 8, Flexner-Jobling rat carcinoma, mouse sarcoma 180, rat sarcoma 10, and a rat fibroma required 300, 1,200, 1,650, 1,950, and 2,800 r respectively to kill half their cells *in vitro*.

For the rat mammary carcinoma R2426, employed in these experiments, Eisen (4), using a 200 kv. machine and a filter of 0.5 mm. of copper, found that between 4,000 and 5,500 r was required to produce death of its cells *in vitro*. About 4,750 r was necessary to kill half of the transplants. The results of the present work show that transplants of this tumor 4 years subsequently to Eisen's determinations failed to regress *in vivo* after treatments of 4,000 r.

No data are available regarding the radiosensitivity of the transplantable mammary carcinoma in C57 mice. Henshaw (10) found that as many as 40 per cent of some strains of mice were killed by exposure of the whole body to 400 r. Preliminary observations showed that a single application of 500 r to a large tumor was sufficient to cause the death within 1 day of some C57 mice. Therefore, only 250 r were tried as a fractional dosage for the first 4 treatments. This dosage was tolerated satisfactorily, but was insufficient to produce regression of the tumors. It is unfortunate that neither the rats nor the mice could tolerate a dosage sufficient to make their tumors recede.

The total elapsed time in making the photometric measurements was 17 days for the carcinomas in rats and 19 for the carcinomas in mice. The measurements on comparable sections do not suggest that there was any appreciable fading of the stain during the course of the measurements.

Since the tumors may vary slightly in their cellular structure from one area to another it is not difficult to understand how, when the mean differences between radiated and nonradiated tumors are so slight, one might select small areas of a radiated tumor with a mean increase in thymonucleic acid as compared with the control. Such readings, then, would not show the decrease found elsewhere. This illustrates the value of averaging the figures for many readings on different sections and from comparable but different tumors.

As indicated by the mean number of cells per area, the cells in the mouse tumor were smaller than those in the rat tumor. The percentage absorptions per area were only slightly greater for the mouse tumors. The mean per cent absorption per cell for all the 62 mouse tumors was 1.56, and for the 48

rat tumors 0.93. This means that the rat carcinoma cells contained approximately only 60 per cent as much thymonucleic acid as those of the mouse tumor. The coefficients of variation, which are an indication of the variation in thymonucleic acid between different volumes of the tumor, were smaller for the mouse tumors than for the rat tumors.

It is recognized that such factors as the blood supply of transplanted tumors may affect their growth. Therefore the attempt was made in these experiments to transplant all tumors into the superficial subcutaneous tissues and not into the deeper tissues or muscles. The control carcinoma for each radiated tumor was selected from a corresponding region on the opposite side of the body of the animal. Each control and radiated tumor was measured in sequence on the same day to facilitate comparisons and reduce possible variations due to the recording apparatus. Since transplantable tumors in different animals, or perhaps in different regions of the same animal, and tumors measured at variable intervals of time are not entirely suited for direct comparison, each radiated tumor was considered in relation to its corresponding control by determination of the ratios shown in Tables II and III.

Thus it is felt that these ratios represent a more significant figure for comparison than the individual series of measurements from which they were derived. Inspection of these ratios for the rat tumors shows that there is a statistically significant decrease in the mean thymonucleic acid content per unit volume in all the 6 rat carcinomas receiving 4,000 r. The tumors receiving only 2,000 r showed more variable results and less reduction in mean thymonucleic acid per area and per cell. The 4,000 r produced a mean reduction of 12.7 per cent and 5.4 per cent in the thymonucleic acid per area and per cell respectively, whereas 2,000 r gave only insignificant changes.

The ratios of absorption per area show that the thymonucleic acid per unit volume was decreased following radiation of the mouse tumors in 15 instances, and unchanged in 1 case. This decrease was statistically significant in 9 instances and the mean decrease of the 16 radiated tumors was significant. There was a mean decrease of 5.3 per cent in the thymonucleic acid content per unit volume of carcinoma following radiation. There was a mean decrease in the thymonucleic acid content per cell of 3 per cent in the mouse tumors. In 4 of 16 sets of tumors the amount of thymonucleic acid per cell was significantly decreased, and no mouse tumor showed a significant increase following irradiation. Since the amounts of radiation used were not large enough to produce definite regression of the tumors, one might expect only the more radiosensitive ones to show a significant change following radiation.

The validity of the results obtained may be examined in another manner. The determination of the mean number of cells per area is fairly accurate. If there was a decrease in the number of cells per area, one would expect an equivalent decrease in the percentage of absorption per area, provided the amount of thymonucleic acid per cell was unchanged. For the mouse tumors, a decrease in cells per area of 2.7 per cent should be accompanied by an equivalent decrease in the amount of thymonucleic acid per cell if the amount per cell remained constant following radiation. The observed decrease in thymonucleic acid per area of 5.3 per cent is not explained by a decrease of 2.7 per cent in the number of cells per area unless the observed decrease in absorption per cell of 3 per cent is also taken into consideration. The observed decrease in the number of cells per area, which was associated with an increased size of the radiated cells, may represent a swelling due to an increased amount of intracellular fluids. This could represent either an increased imbibition of extracellular fluids or a retention of intracellular metabolites.

It is not within the scope of this paper to enter into a discussion of the effects of radiation upon cells in general. It is thought that the most important effects of roentgen rays are on the nuclei. Among the effects most frequently recognized are degenerative changes, pyknosis, pseudoamitosis, and giant nuclei. The abnormal mitosis and inhibition of mitosis are evidenced by clumping, fragmentation, and lagging of the chromosomes which may be associated with disturbances of thymonucleic acid. Cramer (1, 2) has carefully studied the effects of radium upon spontaneous and transplanted mammary carcinomas in mice. With large doses he was able to notice arrest or diminution of growth at the end of 1 week.

Eckert and Cooper (3) studied 21 cases of squamous cell carcinoma of the cervix uteri by means of visual inspection of sections stained by the Feulgen reaction and by hematoxylin and eosin. Five cases were biopsied before, 9 after, and 7 both before and after irradiation. They found that the Feulgen reaction revealed more nuclear damage than was recognized with hematoxylin and eosin staining. The nuclear changes noted were pyknosis, and edema-like swelling of the nucleus, and vacuoles within the nucleus. The nucleic acid seemed to disappear from the swollen nuclei following radiation. Particles of extranuclear material that stain with the Feulgen reaction have been reported by Eckert and Cooper, and by Roskin (16), who also observed vacuoles in some nuclei following radiation.

Of especial interest in relation to the present work are the studies by Mitchell (12-14) on the effects of therapeutic doses of roentgen and gamma radiations on the nucleic acid metabolism of tumors. Mitchell

used 3 methods of analysis: (a) ultraviolet (2537 A) photomicrography with densometric observations on the photographic plates, (b) ultraviolet absorption measurements with a Hilger-Spekker photometer, and (c) a spectroscopic image method. Using combinations of these 3 methods, Mitchell observed a striking increase in the ultraviolet absorption of the cytoplasm of cells after irradiation in 11 of 15 pairs of biopsy specimens from cases treated with gamma radiation, and in 13 of 17 pairs of biopsy specimens from cases treated with roentgen radiation. The order of magnitude of the observed increase corresponded to a concentration in the cytoplasm of the affected cells of 3 per cent of pentose nucleotides. This material, which is thought to be ribonucleotides, was present in considerably more than the normal concentration. The pentose nucleotides were concentrated chiefly about the nuclear membrane.

The changes observed by Mitchell in the nuclear absorption showed great variation, and are insignificant in 3 of the 6 cases. He suggests that some of the apparent increase in nuclear absorption may be caused by the nucleic acids in a rim of overlapping, adjacent cytoplasm. Such an error would not occur with tissues measured after staining by the Feulgen reaction.

Mitchell attributes the increased nucleic acids in the cytoplasm following radiation to either an increased rate of formation or a decreased rate of removal of ribonucleotides. He suggests that there is an inhibition of synthesis of thymonucleic acid and an accumulation of ribonucleotides in the cytoplasm of the radiated cell. These changes may be due to inhibition by radiation of the process of reducing ribonucleotides to desoxyribonucleotides within the nucleus. Such a hypothesis is very interesting, but Mitchell's own observations showed a slight increase, or no change, in nucleic acids within the nucleus instead of an inhibition of synthesis or decrease in the amounts of thymonucleic acid following radiation.

Both the observations of Mitchell and the results reported herewith show that the nucleic acids of tissues do not react uniformly to radiant energy. These measurements of 5,500 areas of tumors containing 126,000 cells gave results in which the thymonucleic acid content of the tumors was decreased to a statistically significant degree following radiation in some tumors, but was increased in a few instances. In general, however, these observations support the belief that large doses of roentgen radiation tend to cause an increase in size of the cells and a decreased amount of thymonucleic acid per cell.

The evidence that malignant cells have an abnormal balance of nucleic acids has been discussed in other publications (18, 20, 21). Although the reason is not evident, most neoplastic cells are more sensitive to the effects of irradiation than the corresponding cell types from which the tumor arose. The suggestion is made that a neoplastic cell, with its disturbance in nucleoproteins, may be killed by further damage to its nucleic acids through radiation more readily than a normal cell.

To the theory suggested by Mitchell these results lend a support that his own experimental work lacked. A decrease in the amount of thymonucleic acid per cell has been demonstrated in some tumors following irradiation. These observations would support the concept that radiation interferes with the formation of desoxyribonucleic acid within the nucleus and initiates a disturbance of nucleic acid metabolism that may lead to death of the cell. The exact mechanism of action of the roentgen rays is unknown although the results of their action on the thymonucleic acid of genes with the production of somatic mutations has been frequently observed. Roentgen rays may produce atomic alterations in the vital thymonucleic acid molecule or in some of the other substances necessary to normal nucleic acid metabolism. The disturbance is indicated by abnormalities of the chromosomes and accumulation of ribose nucleic acids within the cytoplasm of the cell. The increased pentose nucleic acids may explain the swelling of the cells and the increased basophilic staining of cytoplasm that is sometimes observed following radiation.

SUMMARY AND CONCLUSIONS

The relative thymonucleic acid content of transplantable mammary carcinomas in rats and mice was measured by means of the Feulgen reaction and a special microphotometric apparatus. The tumors on one side of the animal were radiated while those on the other side were protected by lead shielding and used as controls. The dosage employed, which varied up to 4,000 r, was insufficient to produce definite regression of the tumors, but growth retardation was evident in many instances.

The 6 tumors in rats that received 4,000 r all showed a significant decrease in the amount of thymonucleic acid per unit volume of tissue, and 2 showed a significant decrease in the amount per cell. These 6 tumors had a mean decrease of 13 per cent and of 5 per cent in their content of thymonucleic acid per area and per cell respectively. The effects of 2,000 r on 6 rat tumors were more variable and less significant.

The 16 radiated mouse tumors showed a mean decrease in thymonucleic acid content per area and per cell of 5 and 3 per cent respectively. In 9 of the 16 the decrease in thymonucleic acid per area was statistically significant, and in 4 of these the decrease per cell was significant.

The results of these experiments would support a hypothesis that roentgen radiation may alter the molecular structure of vital substances within the nucleus and produce a disturbance of the nucleoproteins, which in some instances is followed by death of the cell. Mitchell has found that ribonucleic acids are increased in the cytoplasm following radiation. The present observations, which show a decrease in desoxyribonucleic acid in radiated cells, suggest that one of the most important intracellular effects of roentgen radiation is the production of an upset in the normal balance and metabolism of both types of nucleic acids.

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Chromosomal Enlargement in Neoplastic Rabbit Tissues

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A study of the chromosomes of rabbit neoplasms was undertaken for several reasons. The primary object was to determine whether the enlargement of chromosomes to double or quadruple their original size occurred in malignant tissues of yet another organism, the rabbit, as it had been observed to occur in cancers of the mouse (8), goldfish (2), man (7), and the rat (3). The secondary aim was to learn whether chromosomal enlargement was possibly of value as a morphological indicator of autonomy in tumors.

MATERIALS AND METHODS

The tissue specimens used were kindly provided after fixation by Dr. Harry S. N. Greene, of the Yale University School of Medicine. In addition to the small intestine of an adult female (obtained elsewhere), the normal material included the liver, kidney, and lung of a 24 day old fetus. Among the tumors examined was a transplant of an adenocarcinoma of the uterus of an animal designated X1800-2. This adenocarcinoma was of proved growth autonomy in the anterior chamber of the eye (10). Two other uterine tumors studied, from animals M101-3 and X16072-2, were of doubtful or negative autonomy. That from the latter animal was an adenoma of the endometrium. Examinations were made of the chromosomes of specimens of a breast tumor of animal HA672-2 taken at biopsies on November 11, 1942, on January 7, 1943, and on April 22, 1943, as well as at autopsy after sacrifice on June 16, 1943. According to Dr. Greene (10), this mammary tumor appeared histologically to be cancerous, but it was of negative growth autonomy and autopsy revealed no invasion by it or metastases from it. In the uterus of animal HA672-2, however, there was found at autopsy a primary adenocarcinoma, which grew slowly in some of the anterior chambers to which it was transplanted. The chromosomes of this adenocarcinoma were also investigated.

The specimens were fixed in Carnoy's fluid, composed of 1 part of glacial acetic acid and 3 parts of absolute alcohol. Several days later small bits of the tissues were stained in acetocarmine, spread out under coverglasses, dehydrated in alcohol, and mounted in diaphane.

The acetocarmine preparations were studied under oil immersion, and camera lucida drawings at a magnification of 3,000 times were made of 25 metaphase figures of each specimen in most of the cases. In the mammary tumor at autopsy mitoses were rare and only 10 were drawn; and 30 metaphases were drawn from the adenocarcinoma of animal X1800-2. The average volume of the chromosomes of each metaphase was calculated as previously described (7, 8).

An attempt was made to analyze the nucleoli of resting nuclei in order to determine the probable initial number of plasmosomes before fusion set in. While some data were obtained, the acetocarmine preparations of rabbit tissues unfortunately did not prove to be favorable material for the study of plasmosomes.

OBSERVATIONS

Table I sets forth the frequencies of metaphase figures containing chromosomes of certain average volumes. The 3 embryonic tissues (Figs. 1 and 2) had average chromosome volumes of about 0.4 and 0.5 cubic micron, while the chromosomes of the adult small intestine were slightly larger. The 4 normal tissues had unimodal distributions of metaphases according to average chromosome volume. The maximum number of plasmosomes in the resting nuclei of these normal organs seemed to be 6.

In the uterine adenocarcinoma of animal X1800-2, on the other hand, 3 groups of metaphases according to average chromosome volume could be distinguished (Figs. 7 to 10). Their respective means fell at about 0.5, 1.2, and 2.4 cubic microns. The second group was numerically predominant. It was composed chiefly of diploid division figures, although the 2 chromatids of each metaphasic chromosome were often so widely separated that at first glance many of the figures appeared to be polyploid. Since most of the resting nuclei of the adenocarcinoma had a dozen or slightly fewer plasmosomes, it is probable that the chromosomes of the predominant second

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group of metaphases had twice the normal number of strands. It is also likely that the chromosomes of the one figure in the third group, which averaged 2.4 cubic microns in volume, were constituted of 4 times the normal number of chromonemata. This metaphase is illustrated in Fig. 10. The uterine adenocarcinoma of HA672-2 also seems to have contained chromosomes of double the normal complexity, although in a minority of its dividing cells (Fig. 6).

The apparently nonautonomous uterine tumors of animals M101-3 and X16072-2 did not give evidence of a chromosomal change from the normal condition (Fig. 3). Their average chromosome volume, 0.6 cubic micron, can be regarded as essentially normal, and in the absence of a normal control specimen it can be used as a basis for estimating the expected volume of double chromosomes in the adenocarcinomas.

Polyploidy was found in some of the neoplasms. The diploid number of rabbit chromosomes, it will be recalled, is 44 (14). The breast tumor of animal HA672-2 had 2 approximately tetraploid figures among 85 metaphases, and the uterine adenocarcinoma of this animal had one octoploid figure with small chromosomes among 25 figures examined. Of the 30 metaphases drawn from the adenocarcinoma of X1800-2, 8 were in the tetraploid range with about 70 to 90 chromosomes, and 2 had about 120 chromosomes. One of the latter metaphases is illustrated in Fig. 8. Polyploidy in the cancer of X1800-2 was largely or perhaps entirely confined to metaphases with enlarged chromosomes. We do not consider polyploidy to be of especial significance in cancer, although our earlier concept (8) of endomitosis that could give rise either to polyploidy or to polyteny, depending on the completeness of the reduplication and separation of sister strands, might be construed as suggesting that a tissue in which polyteny was found was also liable to contain instances of mitoses with multiples of the normal number of chromosomes.

TABLE I: FREQUENCIES OF METAPHASES BY AVERAGE CHROMOSOME VOLUME IN RABBIT TISSUES

Average chromosome volume, μ^3	Normal organs				Ut. adenocarcinomas		Ut. adenomas		Mammary tumor HA672-2			
	24 day fetus			Adult								
	Liver	Kidney	Lung	Sm. int.	X1800-2	HA672-2	M101-3	X16072-2	Nov.	Jan.	Apr.	June
0.2-0.3	4	2	6		1							
0.3-0.4	9	2	8	4		1	1				1	3
0.4-0.5	8	8	7	3	1	3	4	3	3	1		2
0.5-0.6	3	9	3	6		9	7	11	4	1	3	3
0.6-0.7	1	4	1	10	2	9	7	9	7	9	13	1
0.7-0.8				1			3	2	4	6	6	1
0.8-0.9				1			3		3		1	
0.9-1.0					3	1			1	1	1	
1.0-1.1					7	1			1	6		
1.1-1.2					3	1				1		
1.2-1.3					6				2			
1.3-1.4					5							
1.4-1.5					1							
2.4-2.5					1							
MEAN CHROMOSOME VOLUMES IN CUBIC MICRONS												
Simple	0.40	0.49	0.38	0.56	0.51	0.56	0.61	0.59	0.65	0.66	0.65	0.49
Double					1.17	1.02			1.11	1.04	0.99	
Quadruple					2.44							

The last 3 of the 4 specimens taken from the probably regressing mammary tumor of animal HA672-2 showed a progressive decrease in frequency of metaphases interpreted as containing double chromosomes. The specimen taken at autopsy was notable for the smallness of its chromosomes. In the first 2 biopsy specimens the bimodality of frequency of metaphases according to average chromosome volume provided additional evidence of the presence of double chromosomes, although no nuclei were noted with more than 6 plasmosomes.

The data on chromosomal enlargement and autonomy in the various rabbit tumors are assembled in Table II. The 4 entries under the percentage of metaphases with enlarged chromosomes in the mammary tumor are those for the 3 biopsy and the 1 autopsy specimens.

DISCUSSION

The rabbit appears to have been among the first organisms in which chromosomal changes of the type under discussion were noted. Barratt (1) in 1907 injected Scharlach R dissolved in olive oil subcutaneously in the rabbit, and in the subsequent extreme hypertrophy of the Malpighian layer of the skin there occurred altered chromosomes. They were coarser than the chromosomes found in the testis, and in a minority of the dividing epidermal cells the

chromosomes were so large that some of them no longer preserved the form of curved rods. In these cells the chromosomes appeared to be in pairs or in undivided pairs. Although the epidermal proliferation subsided when the Scharlach R disappeared, it may be conjectured that Barratt observed a process similar to that later noted (6) in mouse skin painted with a benzene solution of methylcholanthrene. In the latter case, application of the carcinogen was followed in several days by the appearance of enlarged chromosomes in about one-tenth of the dividing cells. A further cytological analysis has indicated that the enlarged chromosomes of the treated mouse skin, as well as those of carcinomas descended from such skin, probably contained a greater number of strands than is normally found (5).

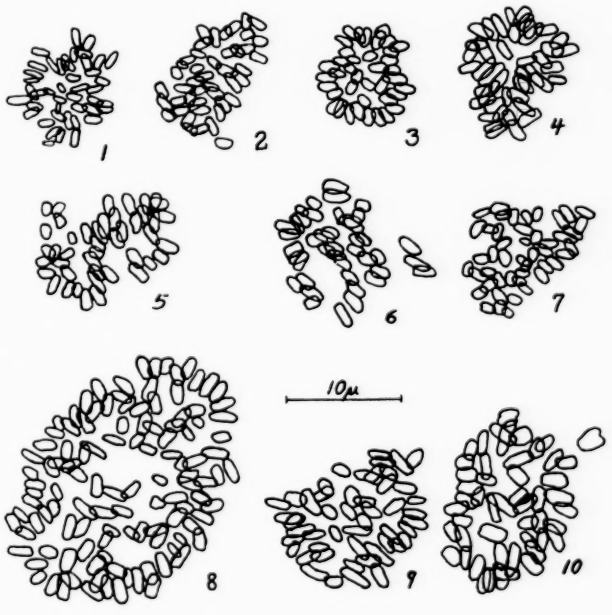

FIG. 1.—Metaphase from fetal rabbit liver; 44 chromosomes of an average volume of 0.4 cubic micron.

FIG. 2.—Metaphase from fetal kidney; 44 chromosomes averaging 0.5 cubic micron in volume.

FIG. 3.—Metaphase from uterine adenoma of rabbit X16072-2; 44 chromosomes averaging 0.6 cubic micron in volume.

FIG. 4.—Metaphase from biopsied mammary tumor of HA672-2; 44 chromosomes averaging 0.8 cubic micron in volume.

FIG. 5.—Metaphase from biopsied mammary tumor of HA672-2; 44 chromosomes averaging 0.7 cubic micron in volume.

FIG. 6.—Metaphase from uterine tumor of HA672-2 at autopsy; 42 chromosomes, possibly double, averaging 1.1 cubic micron in volume.

FIG. 7.—Metaphase from uterine adenocarcinoma of X1800-2; 47 simple chromosomes averaging 0.5 cubic micron in volume.

FIG. 8.—Metaphase from uterine adenocarcinoma of X1800-2; about 117 double chromosomes averaging 1.1 cubic micron in volume.

FIG. 9.—Metaphase from uterine adenocarcinoma of X1800-2; 49 double chromosomes averaging 1.2 cubic micron in volume.

FIG. 10.—Metaphase from uterine adenocarcinoma of X1800-2; 51 quadruple chromosomes averaging 2.4 cubic microns in volume.

The 4 normal rabbit tissues examined showed somewhat different sizes of chromosomes, and Painter (14), moreover, has found spermatogonial chromosomes of the rabbit to be considerably smaller than amniotic chromosomes. Hence it is possible that the rabbit has much the same variability in chromosome size from organ to organ as has been described in the rat (3). Since there was no difference in maximum number of plasmosomes per resting nucleus from one rabbit organ to another in our small series, it may be inferred that the observed differences in size of normal chromosomes were not expressions of polyteny. The larger series of organs studied in the rat (3) permitted the conclusion to be drawn that within a species a certain average chromosome volume was characteristic of a given cell type under given conditions. Chromosomes of several organs in the adult rat, notably the liver and kidney, were considerably larger than their counterparts in the newborn rat. The enlargement of the chromosomes in the adult organs was again not accompanied by changes in plasmosome number, although there were such nucleolar alterations concomitant with the chromosomal changes in neoplastic tissues.

TABLE II: CHROMOSOMAL ENLARGEMENT AND AUTONOMY IN RABBIT TUMORS

Neoplasm	Animal	Metaphases with enlarged chromosomes, %	Autonomy *
Uterine adenocarcinoma	X1800-2	87	Strongly positive
" "	HA672-2	12	Weakly positive
" adenoma	X16072-2	0	Negative
" tumor	M101-3	0	"
Mammary "	HA672-2	16, 32, 4, 0	"

* From H. S. N. Greene (10).

Most of the cells in the adenocarcinoma of animal X1800-2 contained double chromosomes of the type found in cancers of other organisms. Apparently such chromosomes were also present in some of the cells in the uterine adenocarcinoma of HA672-2, and in about one-fourth of the dividing cells of the early specimens taken from the mammary tumor. In the 2 other new growths examined no enlarged chromosomes were to be seen. That these 2 adenomas were also lacking in growth autonomy is of interest.

By reference to Table II it may be seen that although our small selection of tumors yielded little evidence to the contrary, the data are hardly sufficient to justify the assumption of a connection between autonomy of a tumor and the structural condition of the chromosomes in the majority of the cells. This

may likewise have been indicated by the studies of Greene. A consistent relation was not found, for instance, between autonomy and the degree of anaplasia in tumors (12). The relation between anaplasia and cancerous chromosomal change of the sort involved here is itself poorly defined, although Ehrich (9) regarded the generalized doubling and quadrupling of nuclear volume in cancers as an anatomical expression of anaplasia, and we have shown this nuclear volume doubling probably to result from doubling of chromosome strands (7). Furthermore, the positive growth autonomy of embryonic tissues (11) would demand a chromosomal similarity between embryonic and autonomous malignant tissues, if the chromosomes were intimately concerned in autonomy. The embryonic tissues of the rabbit, mouse, and rat subjected to examination have contained uniformly simple chromosomes, which obviously do not agree in point of structure with the double chromosomes of malignant tumors. Although a functional similarity has been suggested for chromosomes of embryonic tissues and cancers of the rat (4), the question of the relation of chromosomes to autonomous growth remains undecided.

We wish to lay greater stress in this paper on the finding of enlarged chromosomes, of the double type seen in other cancers, in the rabbit's uterine adenocarcinoma. The rabbit now becomes the fifth organism proved to have such chromosomes in neoplastic tissue. Three of the 5 organisms are rodents, and it may be expected that cancers of other rodents will be found to be marked by the possession of similar enlarged chromosomes. Four of the 5 organisms are mammals, and all 5 are vertebrates. Among invertebrates, polytene chromosomes are often of normal occurrence, especially in insects. If the structural difference between chromosomes of normal and malignant tissues in the vertebrates is of causal significance in malignancy, then it may be expected that the enlarged chromosomes of vertebrate cancers will prove to be different in some respect from the polytene chromosomes of insect tissues. Possibly chromosomal alterations are also involved in the abnormality of Mottram's carcinogen-treated ciliate protozoa (13). Mottram has found the abnormal ciliates to have a multiple constitution in the possession of 2 or more micronuclei, which are sometimes double or quadruple the normal size, instead of 1 small micronucleus as in the normal controls.

SUMMARY

An adenocarcinoma of the rabbit uterus was found to have a high frequency of metaphases, the majority of which were diploid, containing chromosomes about twice as large as those in normal tissues, and this neoplasm also had about 12 plasmosomes in most of its resting nuclei. Since the 4 normal tissues examined seemed to have a maximum plasmosome number of 6 per nucleus, it is probable that the enlarged chromosomes of the adenocarcinoma contained twice as many strands as the chromosomes of normal tissues. Similar enlarged chromosomes were present in lower frequency in another uterine adenocarcinoma and a mammary tumor, but none was found in 2 apparently benign tumors of the uterus.

Although the evidence is suggestive, the series of tumors examined is too small for definite conclusions to be drawn concerning the relation of chromosomal conditions to growth autonomy.

The rabbit becomes the fifth organism to show a chromosomal enlargement by doubling of strands in its malignant cells.

ACKNOWLEDGMENT

I am indebted to the late Dr. Edgar Allen and to Dr. Harry S. N. Greene for initiating this study.

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Abstracts

Experimental Research, Animal Tumors

Irritation and Carcinognesis. Berenblum, I. [Oxford Univ., Oxford, England] *Arch. Path.*, **38**:233-244. 1944.

A general review, with extensive reference to the literature, in which the author assumes that any effect irritation might have on carcinogenesis is brought about through the reparative hyperplasia that it induces. He points out that every carcinogen that produces a tumor at the site of application or injection is an irritant in the sense that it induces a continued state of reparative hyperplasia. Furthermore, in all cases in which sufficiently accurate observations can be made, it is seen that the primary tumor is preceded by a stage of hyperplasia. From these facts he concludes that hyperplasia is an essential precursor of neoplasia. But it is certain that only some and not all irritants are carcinogenic; therefore, preneoplastic hyperplasia must be a specific type biologically distinct from ordinary reparative hyperplasia.

The author further points out that there is good reason to believe that carcinogenesis is not a single process but consists of several component phases that may be dissociated. He cites evidence that indicates that preneoplastic hyperplasia is a highly specific type of hyperplasia, since only carcinogenic irritants can produce it with certainty, but that, once the preneoplastic state has been induced by a true carcinogen, a benign tumor can be made to appear at that site, and a tumor already present can have its progress to carcinoma hastened by the action of a variety of noncarcinogenic irritants.

Although recognizing the tentative nature of these conclusions, the author deduces certain practical lessons from them: (a) that there is little danger of an ordinary irritant producing a tumor of its own accord; (b) that this applies also to the initiation of a preneoplastic lesion; and (c) that, given a preneoplastic lesion, the subsequent development of a benign tumor at the site may be facilitated, and its progress to cancer hastened, by the action of a variety of nonspecific irritants, though this facilitation is far less effective with most nonspecific irritants than it is with a true carcinogen.—J. G. K.

Zur Beziehung zwischen Konstitution und cancerogener Wirkung aromatischer Kohlenwasserstoffe. [The Relationship between Constitution and Carcinogenic Effect of Aromatic Hydrocarbons.] Lettré, H. [Göttingen Univ., Göttingen, Germany] *Ztschr. f. physiol. Chem.*, **280**:28-31. 1944.

When the polycyclic hydrocarbons are considered as derivatives of anthracene and phenanthrene, those that still possess the symmetry plane going through the center of the middle ring of the two original hydrocarbons are never carcinogenic. Loss of this symmetry by substitution seems to be a necessary, though not sufficient, condition for the carcinogenicity of an aromatic hydrocarbon. This is demonstrated by a series of examples.—Z. D.

Polycyclic Aromatic Hydrocarbons. Part XXIX. Derivatives of 1:2:5:6-Dibenzfluorene. Cook, J. W., and Preston, R. W. G. [Glasgow Univ., Glasgow, Scotland] *J. Chem. Soc.*, 553-561. 1944.

A series of derivatives of 1,2,5,6-dibenzfluorene has been prepared directly from this hydrocarbon and from 1,2,5,6-dibenzfluorenone, in an endeavor to find compounds more inhibitory to tumor growth than the parent hydrocarbon. Two new synthetic routes to derivatives of 1,2,5,6-dibenzfluorene have been devised. 1,2,7,8-Dibenzfluorene has also been prepared, and some possible synthetic routes to the unknown 2,3(2′,1′-naphtha)fluorene have been explored. Biological tests by Haddow will be reported elsewhere.—E. L. K.

Some Observations on the Photochemistry of Fluorescent Substances. Part I. The Quenching of Fluorescence by Nitric Oxide and the Photochemical Formation of Nitroxides. Weil-Malherbe, H., and Weiss, J. [Cancer Research Lab., Roy. Victoria Infirmary, and King's Coll., Univ. of Durham, Newcastle-upon-Tyne, England]. **Part II. Concentration Quenching (Self-quenching) of Fluorescence.** Weiss, J., and Weil-Malherbe, H. *J. Chem. Soc.*, 541-544; 544-547. 1944.

Studies of the fluorescence of 3,4-benzpyrene; 20-methylcholanthrene; anthracene; 1,2-benzanthracene; 9,10-dimethyl-1,2-benzanthracene; 1,2,5,6-dibenzanthracene; naphthacene; rubrene; ethylchlorophyllide.—E. L. K.

Carcinogenic Effect of Aminoazobenzene. Kirby, A. H. M. [Glasgow Roy. Cancer Hosp., Glasgow, Scotland] *Nature*, **154**:668-669. 1944.

Sixteen Wistar rats received a low protein diet similar to that described by Miller and his associates (*Cancer Research*, 1:699. 1941) with the addition of *p*-aminoazobenzene (at first 0.3, later 0.2 gm. per 100 gm. food). One died at 13 months with very small hepatomas, and 2 died at 17 months with large hepatomas (in 1, liver plus tumors weighed 47.8 gm.), which were metastasizing through blood vessels to the mesentery. Hence the methyl groups in N,N-dimethyl-*p*-aminoazobenzene appear not to be essential to this form of carcinogenesis.—E. L. K.

Über das aus krebserregenden Azofarbstoffen entstehende Fermentgift. [Enzyme Poison Arising from Carcinogenic Azo Dyes.] Kuhn, R., and Beinert, H. [Kaiser-Wilhelm Inst. f. Med.-Forsch., Inst. f. Chem., Heidelberg, Germany] *Ber. d. deutsch. chem. Gesellsch.*, **76**:904-909. 1943. Abstr. in *Chem. Zentralbl.*, **II**:1884. 1943.

Kensler, Dexter, and Rhoads (*Cancer Research*, **2**:1. 1942) and Kensler, Young, and Rhoads (*J. Biol. Chem.*, **143**:465. 1942) reported the inhibition of yeast enzyme systems (diphosphopyridine nucleotide system and carboxylase) by *p*-diamine, a phenomenon allegedly related

to the stability of the free radicals arising from the diamine, and to the carcinogenic activity of the corresponding azo dyes. Kuhn and Beinert repeated the tests on carboxylase purified according to the method of Green (*J. Biol. Chem.*, **135**:795. 1940) (Kensler used washed desiccated yeast) with crystalline Wurster salts. N,N-Dimethyl-*p*-phenylenediamine and N,N,N′,N′-tetramethyl-*p*-phenylenediamine were not inhibitory in nonoxidized form. Wurster's Red, Wurster's Blue, and solutions of *p*-diamine oxidized to the same stage (as the Red and Blue dyes) with bromine (1 atom per molecule diamine) were inhibitory. If the solutions were further oxidized with 2 to 4 atoms of bromine per molecule, the inhibition was increased, although no more free radicals can be assumed in such solutions. After oxidation of 1 gm. tetramethyl-*p*-phenylenediamine with bromine (4 atoms per molecule) in aqueous solution, and concentration of the product, the inhibitory substance, in yield of 170 mgm., was identified in the distillate as *p*-benzoquinone. This substance, when pure, was more active in the carboxylase test than all oxidation products of *p*-diamine hitherto investigated. Cysteine and ferrous sulfate inhibited the quinone action. Other free radicals, potassium nitroso-disulfonate, and porphyrindin (*Ber. d. deutsch. chem. Gesellsch.*, **68**:1528. 1935) did not inhibit the yeast enzyme systems. Porphyrexid, despite its higher redox potential, was a weaker inhibitor than *p*-diamine; this indicated that the inhibition was not related to redox potential. Reference is made to previously known enzyme inhibitions or impairments by *p*-benzoquinone, and to the successful attempt by Takizawa (*Proc. Imp. Acad.* [*Tokio*], **16**:309. 1940) to elicit tumors in mice by painting them with this compound. The authors postulate that, through the special affinity of carcinogenic azo dyes or their reduction and cleavage products for certain tissue elements in the animal body, *p*-benzoquinone is continuously renewed at such sites, though it does not continue to appear there when *p*-benzoquinone itself is administered.—M. H. P.

The Effect of Biotin on the Metabolism of Liver Slices from Biotin-Deficient Rats. Summerson, W. H., Lee, J. M., and Partridge, C. W. H. [Cornell Univ. Med. Coll., New York, N. Y.] *Science*, **100**:250-251. 1944.

Biotin added to liver slices from biotin-deficient rats always brought about an increase in the utilization of lactate and hence an increase in the amount of bicarbonate produced (in Ringer-bicarbonate solution). Also there were usually slight increases in the values for oxygen consumption and R. Q. The same results were obtained when pyruvate replaced lactate as substrate, but not when a glucose substrate or a nonnutrient medium was used.

The biotin-deficiency-producing diet given to some of the animals used in these experiments contained "butter yellow." The results obtained with apparently normal (except for low biotin content) livers from these animals were the same as those obtained with biotin-deficient livers from animals receiving no "butter yellow."—R. B.

Triphosphate and Triphosphatase. I. The Enzymatic Hydrolysis of Triphosphoric Acid by Triphosphatase of Tumors and Vegetable Objects. II. Specific Chemical Action of Triphosphates. Roberts, I. S., and Neuberg, C. [Cancer Research Lab., Hebrew Univ., Jerusalem, Palestine, and New York Univ., New York, N. Y.] *Exper. Med. & Surg.*, **1**: 386-401. 1943.

Neutralized sodium triphosphate was hydrolyzed *in vitro* by tissue suspensions of benzpyrene rat sarcoma, Rous chicken sarcoma, spontaneous benign and malignant human tumors, and normal chicken breast muscle, as well as by potato phosphatase and a "taka-phosphatase" from a commercial source. A human carcinoma was the only tissue that hydrolyzed unneutralized sodium triphosphate. Triphosphatase preparations active against neutralized, but not against unneutralized, sodium triphosphate have been obtained from Rous sarcoma and benzpyrene rat sarcoma. The optimum pH of the enzyme is between 5.5 and 6.0 in the absence of magnesium ions. Numerous chemical reactions of sodium triphosphate and triphosphoric acid are described.—M. H. P.

Review of the Nutritive Requirements of Normal Mice for Growth, Maintenance, Reproduction, and Lactation. Morris, H. P. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:115-141. 1944.

An extensive survey of the literature is given, from which it is concluded that in many respects the dietary requirements of the mouse are similar to those of the rat. The early view that mice require a higher protein and salt content in their diet than rats do has not been borne out. The essential fats and amino acids appear to be similar for the 2 species. Thiamine, riboflavin, pantothenic acid, and pyridoxine have been found to be essential for growth, but the adrenal-cortical hemorrhagic necrosis of pantothenic acid deficiency, the acrodynia of pyridoxine deficiency, and the testicular degeneration of vitamin E deficiency, which are seen in rats, have not been observed in mice deficient in these vitamins. In regard to fat-soluble vitamins mice appear to react as rats do except for vitamin E in the case of male mice; the importance of inositol seems to be still in doubt. The requirements for lactation are given. In so far as they have been studied the different inbred strains of mice appear to respond similarly to dietary deficiencies.—R. A. H.

Relationships between Spontaneous Tumors of the Lung and Cutaneous Tumors Induced with Ultraviolet Radiation in Strain A Mice. Blum, H. J. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:89-97. 1944.

Previously reported data on cutaneous tumors induced in strain A mice by means of ultraviolet irradiation were reanalyzed from the standpoint of the relationship between the tumors so induced and spontaneous lung tumors. This analysis showed spontaneous pulmonary tumors to be less frequent in ultraviolet treated animals than in their untreated controls. This finding is cited as evidence against the hypothesis that the mode of action whereby ultraviolet irradiation produces cutaneous tumors is through the production of a permeant carcinogen. The distortion of the curve of cumulative pulmonary tumor incidence in irradiated mice indicates the existence of several factors influencing tumor development in general, and at least one of these factors is similar in the 2 types of tumors considered. It is shown that the reduction of lung tumors by ultraviolet irradiation can be partly, but

not entirely, explained by selection for cutaneous tumors before pulmonary tumors have had time to develop. It is suggested that ultraviolet radiation may reduce pulmonary tumor incidence in some indirect way, possibly by curtailing food intake.—R. A. H.

Blut- und Tumorreaktionen nach intravenöser Verabreichung kurzlebiger radioaktiver Körper der Thoriumreihe. [Reactions in Blood and Tumors after Intravenous Injections of Short-Lived Radioactive Compounds of the Thorium Series.] Deucher, W. G., and Leigh-Smith, A. [Röntgen Inst., Bern Univ., Bern, Switzerland] *Schweiz. med. Wchnschr.*, **73**:1092-1094. 1943.

Because of the short half life of thorium C, the leukopenia resulting in rabbits after injection of 2.5-15 mc.-hr./kgm. is transitory and not fatal. Thorium C is accumulated in the liver, intestine, and kidney, but not in the bones or bone marrow. Thorium B, with a longer half life, produces more severe leukopenia than does thorium C and is stored in the bones and bone marrow as well as in the liver, intestine, and kidney. Disappearance of a skin metastasis occurred in 1 patient with bronchial carcinoma treated with repeated intravenous injections of 0.02 mc.-hr. thorium C/kgm. No benefits were observed in 4 other patients similarly treated for lymphogranuloma, epithelioma, or basaloma. The doses used clinically were too small. However, the low toxicity of thorium C is of interest in connection with future trials of artificial radioactive substances.—Z. D.

Radium Metabolism in Rats, and the Production of Osteogenic Sarcoma by Experimental Radium Poisoning. Evans, R. D., Harris, R. S., and Bunker, J. W. M. [Massachusetts Inst. of Technol., Cambridge, Mass.] *Am. J. Roentgenol.*, **52**:353-373. 1944.

Young adult male rats were given small doses of radium by mouth or by intradermal injection. Oral administration of 25 to 100 μgm. resulted about a year later in a high incidence of osteogenic sarcoma, usually in the vertebrae, with metastases in lung and other organs. The tumors were transplantable. At death 1 to 7% of the initial dose remained in the animal's body, over 90% of this being in the skeleton. A terminal retention of 1 μgm. of radium was sufficient to produce sarcoma. Many of the classical symptoms of radium poisoning, such as occur in human beings, were exhibited by the animals. Methods of measuring the radium content of the rats and of their exhalations and excreta are described. The toxicity of radium in man cannot be predicted from experiments on the rat.—E. H. Q.

A Mammary Fibroma Arising in a Rat upon Prolonged Folliculin Treatment. Picco, A. [Turin Univ., Turin, Italy] *Tumori*, **[2] 17**:32-50. 1943. Abstr. in *Chem. Zentralbl.*, **II**:1104. 1943.

A female rat developed a small nodule in the mammary gland after 9 months of injections with 500 I.U. of follicle hormone (Cristalovar) daily (total dose 129,500 I.U.) in aqueous solution. This mammary fibroma, attributed to the large hormone dosage, grew until the animal died 4 months later.—M. H. P.

Incidence of Mammary Carcinoma in Mice Treated with Estrogen. Effect of the Age at Which the Treatment with Estrogen Begins. Loeb, L., Suntzeff, V., Burns, E. L., and Schenken, I. R. [Washington Univ. Sch. of Med., St. Louis, Mo., and Louisiana State Univ. Sch. of Med., New Orleans, La.] *Arch. Path.*, **38**:52-59. 1944.

Two experiments were carried out to determine the effect of the age period at which the administration of estrogens began on the incidence of mammary carcinoma in mice. In the first experiment, in which also the growth processes in the mammary gland leading to the development of cancer were studied, male mice of strain C3H and strain D received subcutaneous injections of an estrogen, the amount of which was adjusted to the weight of the animal, a mouse weighing 25 gm. receiving 200 rat units weekly. The administration of the estrogen was begun in various groups of mice at different age periods, which were as follows: (a) 2 weeks, (b) 4 to 6 weeks, (c) 1¾ to 2¾ months, and (d) 6 to 7 months. Treatment was continued for 5 months in each group. Mammary carcinoma developed only in groups (a) and (b), the maximum in the incidence of these tumors being reached in group (b). Mice of strains C3H and D behaved similarly in these respects, but in strain C3H the number of tumors in groups (a) and (b) was greater than the number in strain D. Likewise, the general development of the mammary gland tissue was, on the average, further advanced in mice which were bearers of mammary carcinoma than in mice not bearing tumors of this type, and it was somewhat further advanced in strain C3H than in strain D. In the second experiment similar results were obtained in strain C3H, although certain factors complicate the interpretation of the observations in this experiment. It was found that in female mice, in contrast to male mice, there was no significant difference in the incidence of mammary carcinoma in the different age groups. Differences in the growth energy or in the readiness of sensitization in young and old tissues and antagonism between male and female sex hormones are considered as possible causes of the observed age effects in the development of mammary carcinoma in mice.—Authors' summary. (J. G. K.)

Mouse Leukemia. XII. The Role of Genes in Spontaneous Cases. MacDowell, E. C., Potter, J. S., and Taylor, M. J. [Carnegie Inst. of Washington, Cold Spring Harbor, N .Y.] *Cancer Research*, **5**:65-83. 1945.

With the increasing knowledge of active agents related to tumors and separable from genes, the question of the role of genes in the apparent hereditary transmission of tendencies to spontaneous malignant growths becomes more critical. If genes are involved, the individuals in a generation in which the reassortment of genes takes place should have genetically diverse tumor tendencies. To demonstrate such genetic diversity, breeding tests of individuals are necessary.

Females from the inbred, low leukemia strain StoLi were mated, (a) with a single male from the inbred, high leukemia strain C58, (b) with 7 of the F_1 sons, and (c) with 50 of the backcross grandsons. Each of the 50 backcross males was described in terms of the incidence of spontaneous leukemia among approximately 50 of its second backcross offspring (total, 2,677 diagnosed autopsies). With the aid of nurses from another, presumably low leukemia strain, all these mice were raised

in the same season of the same year. The incidence of leukemia in these 50 families ranged from 0 to 42.8% with a mode at 17 to 20%.

This variation between families far transcends the variation caused by certain extrinsic influences not equalized by the experimental procedure, and constitutes evidence that the fathers of the first backcross were genetically diverse, as the result of the segregation of genes influencing the incidence of leukemia.

The study of other variables revealed a complex interrelationship of influences modifying the length of life, with differing effects upon the incidence of leukemia, as indicated in the following interpretation. One influence, which varied according to the backcross father, lengthened the lives of leukemic and negative mice without changing the incidence of leukemia. Nurses of one strain lengthened the lives of male negatives and raised the incidence of leukemia among males. This same nurse influence, acting on leukemic mice, lengthened the lives of both males and females, especially those that early manifested the disease, without thereby changing the incidence of leukemia. Femaleness lengthened life by resisting certain nonleukemic causes of death, thereby increasing for females the opportunity for potential leukemics to appear. But the sex difference in incidence was less consistent than the sex difference in length of life of negatives, suggesting that femaleness, to a smaller extent, also resisted leukemia. The increasing parturition age of the mother (low leukemia strain) progressively delayed the appearance of leukemia, without influencing other causes of death, with the result that more and more of the potential leukemics died from other causes, and the incidence of leukemia fell as the mother's age increased. Youngest mothers revealed most accurately the potential leukemics, and the evidence of genetic differences between the second backcross families depends on young mothers; the oldest mothers virtually eliminated leukemia, whatever the genetic tendencies of the family.—Authors' abstract.

The Inheritance of Cancer in Mice. With Special Reference to Mammary Carcinoma. Miller, E. W., and Pybus, F. C. [Roy. Victoria Infirmary, Newcastle-upon-Tyne, England] *Cancer Research,* **5**:84-93. 1945.

The incidence of mammary carcinoma was investigated in a series of crosses and backcrosses between Simpson (high tumor) and Edinburgh (low tumor), and Simpson and CBA (low tumor) strains of mice.

The results confirm the existence of a maternal extrachromosomal factor for mammary carcinoma discovered by previous workers, and also show that an important part is played by inter-strain genetical differences in susceptibility.

No extrachromosomal factor was observed in the inheritance of lung tumors, hepatomas, or bone tumors, while the evidence was inconclusive in the case of lymphadenopathy.—Authors' summary.

The Effect of Foster Nursing on the Incidence of Spontaneous Mammary Carcinoma in Two Inbred Strains of Mice. Miller, E. W., and Pybus, F. C. [Roy. Victoria Infirmary, Newcastle-upon-Tyne, England] *Cancer Research,* **5**:94-101. 1945.

When females of the Simpson (high mammary tumor) strain were fostered by mice of Strong's CBA (low mammary tumor) strain, the incidence was reduced from 69.5% (average age 14.5 months) to 55% (average age 12.4 months). There was unavoidable delay in fostering some of the litters, and even some of those fostered within 24 hours after birth had probably been suckled by their own mothers.

There were fewer mammary tumors in the offspring of the fostered Simpson mice, partly because of a greatly increased mortality from lymphadenopathy. The offspring of fostered tumorous females had a higher tumor incidence than the young of fostered nontumorous mice.

The mammary tumor incidence in CBA females fostered by Simpson mice was raised from 5% (average age 27.8 months) to an average of 44.4% (average age 20.3 months) in all those dying over the age of 6 months, and to 77.8% in those fostered within 24 hours and dying over the age of 10.5 months.

The CBA fostered females were able to absorb the milk influence and transmit it to their offspring even when they themselves died nontumorous; tumors appeared in the young of nontumorous as well as of tumorous fostered females.

There was a very great increase in lymphadenopathy in fostered Simpson mice and their offspring; there was also a definite though smaller increase in the disease in fostered CBA mice and their descendants.

Fostering had no effect on the incidence either of lung adenomas or of hepatomas, except in so far as mice developed mammary carcinomas before reaching the normal lung or liver tumor age.

When young CBA mice had spent from 6 to 12 days with their own mothers before being fostered by Simpson females, they were no longer susceptible to the action of the milk influence, although they were able to transmit it to their young. Possible reasons for this are discussed.—Authors' summary.

Influence of the Milk Factor on the Incidence of Breast Cancer Induced by Oestrone. Dmochowski, L., and Gye, W. E. [Lab. of Imp. Cancer Research Fund, London, England] *Brit. J. Exper. Path.,* **25**:115-118. 1944.

The authors investigated the influence of the milk factor on the development of mammary cancer in "S" low cancer strain and RIII high cancer strain mice nursed by their own mothers, and in their litter-mates foster-nursed by high and low cancer strain mothers, after administration of estrone. The results confirm the statement that the milk of high cancer strain female mice increases the susceptibility to the development of mammary cancer whether of spontaneous origin or induced by estrone.—R. J. L.

Transmission of the Mammary Tumour Inciting Factor by Splenic Grafting. Dmochowski, L. [Lab. of Imp. Cancer Research Fund, London, England] *Brit. J. Exper. Path.,* **25**:119-120. 1944.

Female mice of the "S" low cancer strain were foster-nursed by mothers of the high cancer strain RIII in order to supply them with the mammary tumor-inciting factor. The spleens were removed from the young "S" mice at the time of weaning, and injected into young susceptible hybrids bred from "S" strain mothers and RIII

fathers. Of these hybrids, the females were forcibly bred and the males painted with estrone. Fifty-seven per cent of the former, and 54% of the latter developed mammary tumors. No tumors occurred in control hybrids, but the incidence of mammary cancer in hybrid males painted with estrone was 5% and in forcibly bred females 6%. It is concluded that the spleen of "S" low cancer strain mice foster-nursed by high cancer strain RIII females contains enough mammary tumor-inducing factor to produce mammary cancer in susceptible mice.—R. J. L.

La vitesse de sédimentation des globules rouges chez la souris normale et cancéreuse. [The Rate of Sedimentation of Red Blood Cells in Normal and Cancerous Mice.] REGAMEY, J. *Schweiz. med. Wchnschr.*, **73**:1095-1098. 1943.

Sedimentation rates were measured on 15 cu. mm. of blood according to the method of G. Joyet (*Arch. de physique biol.*, **15**:220. 1939). Values are given for 275 normal males, 68 normal females, 66 mice with diseases other than cancer, 123 with induced cancer, 9 with transplanted mammary adenocarcinoma, and 129 with spontaneous epithelioma. Cancer and other diseases tended to accelerate sedimentation, but there was no constant relation between tumor development and sedimentation rate. —Z. D.

Further Studies on the Quantitative Determination of the Growth of a Transplantable Mouse Adenocarcinoma. REINHARD, M. C., GOLTZ, H. L., and WARNER, S. G. [State Inst. for Study of Malig. Dis., Buffalo, N. Y.] *Cancer Research*, **5**:102-106. 1945.

The daily growth rate of the transplantable Marsh-Simpson adenocarcinoma in mice of the Marsh-Simpson strain was determined by inoculating known numbers of viable tumor cells into 1,546 mice. Fifty-three suspensions varying in concentration from 590×10^3 to 0.08×10^3 viable cells per inoculum were used. The average growth exponent μ was calculated from the growth curves for each suspension and was found to be 0.373 per day. As the number of viable cells inoculated decreased there was a decrease in the percentage of animals that grew the tumor, and at the same time an increase in the latent period was observed.—Authors' abstract.

The Effect of Subcutaneous Injection of Individual Amino Acids upon the Appearance, Growth, and Disappearance of the Emge Sarcoma in Rats. BEARD, H. H. [Louisiana State Univ. Sch. of Med., New Orleans, La.] *Exper. Med. & Surg.*, **1**:123-135. 1943.

An inhibitory action against the Emge sarcoma in rats was exerted by amino acids injected subcutaneously into the abdomen in doses of 18 mgm. in 3 cc. physiological saline daily for 2 weeks, then every other day for 3 weeks, from the day of abdominal transplantation of the sarcoma. In order of decreasing antisarcomatous activity, the amino acids employed were: arginine+histidine; arginine; lysine; phenylalanine; valine; tryptophan; alanine; methionine; histidine; leucine; norleucine; isoleucine, cystine, serine (these 3 were equally active); aspartic acid; proline; glutamic acid; glycine; cysteine; hydroxyproline; threonine. Tumor growth was 3 times faster in the controls than in the rats injected with amino acids. Of the 349 tumors in the injected animals, 47% disappeared, as contrasted with 2.6% of the 280 control tumors. Photographs; tables.—M. H. P.

Effect of Administering Individual Amino Acids and a Casein Hydrolysate upon the Appearance, Growth, and Disappearance of the Emge Sarcoma in Rats. BEARD, H. H. [Louisiana State Univ. Sch. of Med., New Orleans, La.] *Exper. Med. & Surg.*, **1**:136-142. 1943.

When rats were given amino acids (phenylalanine, alanine, valine, leucine, glutamic acid, proline, or arginine+histidine, subcutaneously, or cystine orally) by the technic previously described (*Exper. Med. & Surg.*, **1**:123. 1943) daily for 5 weeks from the day of transplantation of Emge sarcoma, 74% of the sarcomas disappeared, while only 2.4% disappeared in the controls. When the amino acid administration was started 2 weeks after transplantation, only 8.5% of the tumors disappeared. The incidence and growth of the tumors were less in the treated animals of both experiments than in the controls. No effect on the tumor was exerted by injecting 200 mgm. or feeding 3 gm. casein digest (Amigen) daily for 1 month from the day of transplantation. Photomicrographs.—M. H. P.

Inhibition of Tumour Growth. THOMPSON, J. H., HOLT, P. F., and CALLOW, H. J. [Hosa Research Lab., Sunbury-on-Thames, England] *Nature*, **151**:364-365. 1943.

The rate of growth of the Twort carcinoma in mice was considerably decreased by the incorporation of 0.6% ammonium chloride in the drinking water. Ammonium acetate was less effective than the chloride, and ammonium lactate accelerated rather than retarded tumor growth. The inhibiting effect of ammonium chloride and lactate on the carcinoma was not due to any effects on tissue fluid content or body growth. The efficacy of various substances, *e.g.* tissue and urine extracts, previously reported to be tumor inhibitors, may have been due to acidic salts present in them. Ammonium chloride has been used with H 11 in clinical cases of advanced inoperable cancer [results not stated].—M. H. P.

The Effect of Colchicine and X-Rays on Onion Root Tips. LEVINE, M. [Montefiore Hosp., New York, N. Y.] *Cancer Research*, **5**:107-119. 1944.

This report deals with the combined effect of a 0.01% aqueous solution of colchicine and x-irradiation on the root tips of *Allium cepa*, var. *Yellow Globe* and *A. cepa* var. *Brigham Yellow Globe*. Fifteen series of experiments were made, each using from 6 to 40 bulbs. The gross effect of colchicine was studied and checked with microscopic examinations after periods of exposure varying from 6 to 200 hours.

The root tips were studied in the gross and microscopically especially after 18, 24, 36, 48, 72, and 120 hours of exposure to colchicine, followed by exposures to 1 of 3 doses of x-rays (900 r, 1,500 r, and 3,000 r). The combined effects were observed shortly after irradiation and at various periods after the return of the bulbs to water. Bulbs untreated, colchicinized, or x-rayed only, were used as controls.

The effect of colchicine for more than 48 hours, coupled with 900 r, prevented growth of the root tip; exposure to colchicine for 48 hours with 1,500 r induced similar results. With the shorter exposures to colchicine (18, 24, 36 hours) and 1,500 r, the hypertrophied tips produced only a limited growth, which became arrested 5 to 7

days after their return to water. Exposures of 48 hours combined with 3,000 r prevented further growth.

Irradiation with 900 r, 1,500 r, and 3,000 r impaired growth; the time for recovery was proportionately less than that required for the combined effects of colchicine and x-rays.

The leaves of bulbs colchicinized and irradiated with 1,500 r or 3,000 r were retarded in subsequent growth as compared with those x-rayed only. Bulbs colchicinized only showed leaf growth approximately equal to the untreated plants. The combinations of an exposure of 900 r after a treatment with colchicine for less than 72 hours, or 1,500 r and 48 hours of colchicine, were most effective in arresting growth of fundamental plant tissues such as the root tip of the onion.

It is suggested that more intensive studies be made with this drug, combined with x-rays, on tumors of animals and the human subject. The fact that some animal tumors and simple but normal plant structures show delayed growth after such treatment makes it necessary to obtain a concentration of colchicine, coupled with a selective dose of x-rays, that will tend to inhibit or destroy cancerous tissue without injuring the host.—Author's summary.

The Effect of Tumor Extracts on the Growth of Cells in Vitro. Doljanski, L., Hoffman, R. S., and Tenenbaum, E. [Cancer Research Lab., Hebrew Univ., Jerusalem, Palestine] *Growth,* **8**:13-31. 1944.

The growth of chicken fibroblasts *in vitro* was stimulated by Rous chicken sarcoma extract somewhat less than by normal adult chicken heart muscle extract, and was stimulated by dog lymphosarcoma extract somewhat more than by normal adult dog heart extract. An inhibiting action upon fibroblast growth *in vitro* was exerted by rat and hamster sarcoma extracts. The adult rat and hamster differ from most other animals in that all their organs and tissues except brain yield extracts that do not stimulate, and that often inhibit, cell growth *in vitro.* It is concluded that tumor extracts resemble extracts of normal tissues of the same species in their effect on cell growth *in vitro.*—M. H. P

A Selective Lethal Effect of Penicillin on Sarcoma Cells Growing with Normal Tissue in Roller Tube Cultures. Cornman, I. [Wistar Inst., Philadelphia, Pa.] *J. Gen. Physiol.,* **28**:113-118. 1944.

An agent present in pharmaceutical sodium penicillin solutions in 0.85% sodium chloride, passed through a Seitz filter, was found to exert a damaging or lethal effect on sarcoma cells growing in tissue culture. Normal cells growing in the same tubes with the sarcoma cells were either undamaged or damaged less severely than were the sarcoma cells. This selective effect of the penicillin preparations held for 7 sarcomas: King A rat sarcomas 11, 89, 104, 120, 132; Wistar rat sarcoma 304; and C57 black mouse sarcoma 350. In the case of 1 mouse sarcoma (Bagg albino 37) the damaging effect on the tumor was not significantly greater than it was on normal cells. The sodium penicillin preparations used contained impurities which may have been responsible for the effect on sarcoma cells (*cf. Science,* **100**:314. 1944; *Cancer Research,* **5**:126. 1945).—R. B.

The Adrenal Lipids of Mice with High and Low Mammary Gland Tumor Incidences. Vicari, E. M. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] *Anat. Rec.,* **86**:523-543. 1943.

Histochemical determinations revealed: (a) large amounts of adrenal cortical lipids in the low mammary cancer strains C57 black, N, and ce; (b) small amounts in the high mammary cancer strains C3H and dba; and (c) intermediate amounts in strain A, which has a low incidence of mammary cancer among virgin females and a high incidence among breeding females. In an experiment restricted to dba mice it was found that the amount of adrenal cortical lipids was increased after administration of theelin. Accessory adrenal glands were found most frequently in C57 black mice, and least frequently in dba mice.—R. B.

Histologic Changes in the Adrenal Glands of Tumor-Bearing Mice. Dalton, A. J., and Peters, V. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **5**: 99-109. 1944.

The adrenal cortices of mice of several strains having spontaneous or transplanted tumors were studied histologically to determine the effect of the neoplasm upon the lipid content of this gland. Primary tumors were found to be unsatisfactory for the study because of the large number of variables encountered. The cortices of mice bearing transplanted tumors often showed a decrease in lipid stainable with osmic acid, beginning at the inner border of the zona fasciculata and extending, in the more striking cases, to the zona glomerulosa. The degree of lipid depletion was not correlated with any single factor in all neoplasms studied; it appeared, however, to be related to the amount of tumor necrosis in the case of epidermal tumors. In transplanted sarcomas no correlation was evident with either the amount of necrosis or the tumor size, while in mice with inoculated generalized leukemia the lipid depletion was usually rather severe. The possible mechanisms producing this cortical lipid depletion were discussed, and it was suggested that some of the symptoms of cachexia accompanying late malignant neoplastic disease may be the result of adrenal cortical insufficiency.—R. A. H.

Behavior of Ultimobranchial Tissue in the Postnatal Thyroid Gland: The Origin of Thyroid Cystadenomata in the Rat. Van Dyke, J. H. [Washington Univ. Sch. of Med., St. Louis, Mo.] *Anat. Rec.,* **88**:369-391. 1944.

Ultimobranchial cysts lined by stratified squamous epithelium were found in the thyroid glands of albino rats. They occurred most frequently in females and in old rats, and were practically always located in atrophic or degenerating thyroids. No tumors were found in rats 650 days old or younger, but 8 of 16 old rats (801 to 906 days of age) had adenomatous tumors in the thyroid. Seven of these animals were females and 1 was a male. All tumors were found in atrophic thyroid glands, and all but 1 were closely associated with or attached to ultimobranchial cysts. From the histological and cytological evidence it was concluded that the tumors originated from certain densely staining, atypical, gland-like cells derived from the ultimobranchial cysts.—R. B.

Clinical and Pathological Reports

Heredity

Auffallend geringe Bedeutung der "Belastung mit Krebs" bewiesen durch das sehr häufige Freibleiben der Nachkommen aus 121 Ehen krebskranker Gatten im Kanton Glarus. [Surprisingly Small Importance of the Hereditary Factor in Cancer as Indicated by Very Frequent Absence of Cancer in the Progeny of 121 Cancerous Married Couples in the Canton Glarus.] Hanhart, E. [Med. Klin. Zurich, and Kantonale Krankenanstalt Glarus, Switzerland] *Schweiz. med. Wchnschr.*, **73**:446-449. 1943.

The 121 married couples of which both partners had cancer had 590 children, of whom 359 were more than 40, and 286 more than 50, and 188 more than 60 years old. Cancer was found in only 43 of the offspring; these were progeny of 30 of the couples. Of the 286 progeny more than 50 years old, only 38 (13.28%) had cancer, as contrasted with the cancer expectancy of 21% among persons over 45 years old in Zurich. Cancer occurred in only 15.46%, and cancer of the stomach in 11.34%, of the 97 offspring, aged 40 or more, of 33 couples of which both partners had cancer of the stomach.—Z. D.

Hereditary Hemorrhagic Telangiectasia. Report of Case with Review of the Literature. Barrock, J. J. [Milwaukee, Wis.] *Wisconsin M. J.*, **43**:805-814; 888-892. 1944.

An extensive review with 121 references, and a case report with family history.—M. H. P.

Familial Incidence of Tumors of the Brain. Cerebellar Hemangioblastoma. Grossman, M. O., and Kesert, B. H. [Vet. Admin. Facility, Hines, Ill.] *Arch. Neurol. & Psychiat.*, **52**:327-328. 1944.

Case reports. Tumors of the brain occurred in 4 members of one family. In 2, the tumor was a cerebellar hemangioblastoma. The type of tumor was not determined in the other 2, but a cerebellar tumor that could not be removed was present in 1 of them.—M. E. H.

Oral Cavity and Upper Respiratory Tract

Neck Dissections for Metastatic Carcinoma. Brown, J. B., and McDowell, F. [Washington Univ. Sch. of Med., St. Louis, Mo.] *Surg., Gynec. & Obst.*, **79**:115-124. 1944.

A description of surgical technics, with evaluation of results. Various types of carcinomas of mouth, lips, tongue, and other parts of the oral cavity are included.—J. G. K.

Intra-Oral and Laryngeal Carcinoma. A Round Table Discussion (In Part Only). By the Directors of the Ontario Cancer Centers. McCormick, N. A., Chairman. [Windsor, Canada] *Canad. M. A. J.*, **50**:556-562. 1944.

During the period from 1934 to 1939, a total of 464 cases of intraoral (tongue, buccal mucosa, alveolus, floor of the mouth) cancer and 74 cases of carcinoma of the larynx were accepted for treatment at the Ontario Cancer Centers. These represented 5.8% of all new cancer cases treated. Eighty-five per cent of the cases were in males. Results of treatment are discussed.—M. E. H.

Optic-Nerve Atrophy in Malignant Nasopharyngeal Tumors. Folk, M. R. [Chicago, Ill.] *Am. J. Ophth.*, **27**:373-380. 1944.

These tumors seldom invade the optic nerve as its dural sheath is dense and resistant. Compression by the tumor results in optic nerve atrophy. A case with autopsy findings is reported.—E. C. R.

Salivary Glands

Surgical Treatment of Tumors of the Salivary Glands. Janes, R. M. [Univ. of Toronto, and Toronto Gen. Hosp., Toronto, Canada] *Surg. Clin. North Am.*, **23**:1429-1439. 1943.

The radical excision technic (Janes, R. M., *Canad. M. A. J.*, **43**:554. 1940; *Cancer Research*, **1**:838. 1941) has been applied during the past 9 years to 25 benign parotid tumors, with only 1 instance of accidental division of the facial nerve; there were no recurrences in the 21 cases followed. A small malignant mixed tumor, believed benign at the time of operation and removed with surrounding normal gland, has not recurred during 6 years. Of 3 malignant tumors treated by total parotidectomy, 2 recurred. In 2 other instances in which enormous lesions involved the main trunk of the facial nerve, it was necessary to sacrifice the whole nerve; the tumor recurred in 1 case. Parotid tumors should be irradiated for palliation only if surgical removal is impossible. Postoperative radiation is probably indicated for malignant tumors, especially carcinoma. Illustrations.—M. H. P.

Gastrointestinal Tract

Metastasis to Bone as the First Symptom of Cancer of the Gastrointestinal Tract. Report of Three Cases. Bertin, E. J. [Misericordia Hosp., Philadelphia, Pa.] *Am. J. Roentgenol.*, **51**:614-622. 1944.

Reports of 3 cases are presented in which metastases to bone were the first symptoms of cancer in the gastrointestinal tract. In one the metastasis was in the skull, the primary lesion an adenocarcinoma of the sigmoid; in the second a destructive lesion in the fibula was secondary to adenocarcinoma of the stomach; in the third, metastasis in a rib was due to a primary adenocarcinoma of the sigmoid.—E. H. Q.

Diagnostic Value of Blood Studies in Malignancy of the Gastrointestinal Tract. Bolen, H. L. [Fall River Gen., and St. Anne's Hosp., Fall River, Mass.] *Am. J. Surg.*, **63**:316-323. 1944.

An attempt is made to correlate the presence of cancer and the appearance of the blood pattern in a drop of blood on a glass slide.—W. A. B.

Primary Carcinoma of the Jejunum and the Ileum. Boman, P. G. [Duluth Clin., Duluth, Minn.] *Ann. Int. Med.*, **20**:779-788. 1944.

Clinical discussion based upon an analysis of 7 cases.—J. G. K.

Carcinoid Tumour of the Ileum with Metastases in the Mesenteric Lymph Nodes. Stevenson, W. O., and Blanchard, A. J. [Hamilton, Canada] *Canad. M. A. J.*, **51**:259-260. 1944.

A case report. This is another instance to be added to the growing list of argentaffinomas or carcinoid tumors that exhibit malignant properties by producing metastases.—M. E. H.

Carcinoid Tumors of the Ileum (Argentaffinomas). McLeod, C. E. [Middlesex Hosp., Middletown, Conn.] *Am. J. Clin. Path.*, **14**:301-303. 1944.

Report of 2 cases, both with symptoms of intestinal obstruction and metastases in regional lymph nodes.—J. G. K.

Some Complications in the Surgical Handling of Carcinoma of the Left Colon and Rectum. Saltzstein, H. C., and Kelly, J. [Harper Hosp., Detroit, Mich.] *Surg., Gynec. & Obst.*, **79**:27-36. 1944.

Practical considerations.—J. G. K.

Über die Altersregel der Geschwulstentwicklung und die Geschlechtsregel der Geschwulstform. [The Influence of Age Upon Tumor Development and of Sex upon Tumor Form.] Feyrter, F. [Graz Univ., Graz, Austria] *Ztschr. f. Krebsforsch.*, **54**:55-66. 1943. Abstr. in *Chem. Zentralbl.*, **II**:1718. 1943.

Relationships are traced between age, sex, tumor development, and tumor form in benign and malignant, epithelial and mesenchymal, growths of the human gastrointestinal tract.—M. H. P.

Liver

Primary Neoplasms of the Liver. Warvi, W. N. [Univ. of Cincinnati Coll. of Med., and Cincinnati Gen. Hosp., Cincinnati, Ohio] *Arch. Path.*, **37**:367-382. 1944.

Review of the literature, analysis of 36 cases, and discussion.—J. G. K.

Primary Carcinoma of the Liver with Metastases to Bone. Report of a Case. Neumann, M. A. [St. Elizabeths Hosp., Washington, D. C.] *Am. J. Path.*, **20**:895-909. 1944.

The architecture of the primary growth was reproduced in the distant metastases, which were composed of anastomosing cords of epithelial cells, separated by spaces lined with endothelium. In the areas of better differentiation there was evidence of the formation of bile.—J. G. K.

Benign Papilloma of the Ampulla of Vater. Grove, L., and Rasmussen, E. A. [Emory Univ. Med. Sch. and Emory Univ. Hosp., Atlanta, Ga.] *Am. J. Surg.*, **64**:141-143. 1944.

Case report.—W. A. B.

Leukemia, Lymphosarcoma, Hodgkin's Disease

Burn Trauma Precipitating Acute Leukemia or a Leukemoid Condition. Weiss, D., and Haines, K. E. [Station Hosp., Fort Totten, N. Y.] *Am. J. M. Sc.*, **208**:490-494. 1944.

Report of a case, and discussion. The autopsy findings as given do not permit a decision whether the leukocytosis, which became progressively greater during the last 10 days of the patient's life and finally reached a figure of 137,000, was a manifestation of acute leukemia or whether it represented a leukemoid reaction. The role of trauma as a possible etiological factor of leukemia is discussed.—J. G. K.

Eosinophil Leukemia. With Report of a Case. Friedman, M., Wolman, I. J., and Tyner, H. H. [U. S. Marine Hosp., Norfolk, Va.] *Am. J. M. Sc.*, **208**:333-342. 1944.

Clinical and necropsy findings are given, and reference is made to 12 somewhat similar cases in the literature.—J. G. K.

Effect of Vitamin C on the Blood Picture of a Patient with Leukemia. van Nieuwenhuizen, C. L. C. [Utrecht, Netherlands] *Nederl. tijdschr. v. geneesk.*, **87**:896-903. 1943. Abstr. in *Chem. Zentralbl.*, **II**:334-335. 1943.

When ascorbic acid was given intravenously in doses of 500 mgm. to a patient with myeloid leukemia, the leukocyte count decreased from 235,000 to 67,500 [per cu. mm.] although there was a transitory increase in the number of lymphocytes and a mild eosinophilia. The immature forms were not suppressed, in contrast to the results of x-ray therapy. The hemoglobin content and erythrocyte count increased, the bone marrow showed intensified erythropoiesis, and the patient felt better for several months. In a patient with lymphatic leukemia, the same therapy produced temporary reduction in the leukemia without subjective improvement; a single irradiation of the spleen in this subject reduced the lymphocyte count from 500,000 to 14,000.—M. H. P.

Lymphoblastoma in Children Under Thirteen Years of Age. Kaplan, I. I. [Bellevue Hosp., New York, N. Y.] *J. Pediat.*, **25**:155-160. 1944.

A report of 27 young patients with lymphoblastoma. Of these, 10 had leukemia, 11 had Hodgkin's disease, and 6 had lymphosarcoma. The suggestion is made that greater attention to supportive therapy and a more gradual over-all irradiation might prove of greater benefit than the present mode of treatment. No other method offers any more optimistic outlook at present.—M. E. H.

Relationship Between the Lymphoblastic Tumor and the Digestive Tract. Borak, J. [New York, N. Y.] *Am. J. Digest. Dis.*, **11**:241-244. 1944.

A brief description is given of symptoms and findings on x-ray examination of 5 patients with Hodgkin's disease or lymphosarcoma affecting various parts of the alimentary tract. All patients experienced at least temporary improvement following radiation therapy.—E. E. S.

Ichthyosiform Atrophy of the Skin in Hodgkin's Disease. Report of a Case, with Reference to Vitamin A Metabolism. Glazebrook, A. J., and Tomaszewski, W. [Dept. of Clin. Med., and Polish Sch. of Med., Edinburgh, Scotland] *Arch. Dermat. & Syph.*, **50**:85-89. 1944.

A man with Hodgkin's disease was found to have a severe degree of impairment of the liver together with a decided disturbance of vitamin A metabolism. He also showed a generalized ichthyosiform atrophy of the skin, and it is suggested that this atrophy may have been a secondary effect of the vitamin A upset.—Authors' summary.

Hodgkin's Disease with Terminal Miliary Tuberculosis. SIMON, S. M. [Vet. Admin., Bay Pines, Fla.] *M. Bull. Vet. Admin.*, **21**:97-98. 1944.

A case report.—M. E. H.

Hodgkin's Disease with Marked Eosinophilia. WILLIAMS, W. S., and NEUBUERGER, K. T. [Univ. of Colorado Sch. of Med., Denver, Colo.] *Rocky Mountain M. J.*, **41**: 320-322. 1944.

Report of an unusual case of Hodgkin's disease with autopsy findings. There were three uncommon features in the case: marked leukocytosis, a high percentage of eosinophils, and the occurrence of the disease in an elderly female.—M. E. H.

SPLEEN

Untersuchungen über Krebsmetastasen. V. Mitteilung. Die sog., "antiblastische Funktion" der Milz. [Investigations on Tumor Metastases. V. The So-called Antiblastomatogenic Function of the Spleen.] WALTHER, H. E. [Pathol. Inst., Zurich Univ., Zurich, Switzerland] *Schweiz. med. Wchnschr.*, **73**:907-909. 1943.

In 2,842 patients who died of cancer, the average weight of the spleen did not differ significantly from normal. This is seen, also, among animals with spontaneous and induced tumors. The enlargement of the spleen previously observed in animals with transplanted tumors may have been due to a concomitant virus infection. The frequency of primary and secondary tumors in the human spleen is not below expectation, if the mesenchymal character of the organ and its relative weight as compared with bone marrow and liver are considered. The frequency of metastases per unit of weight of organ was in the author's material about the same for spleen as for bone marrow and liver when cases with tumors in the region of the portal vein were excluded. The role of the spleen as a defensive organ against tumors appears therefore very doubtful.—Z. D.

Tumors of the Spleen. HERRMANN, S. F. [Tacoma, Wash.] *Northwest Med.*, **43**:14-17. 1944.

A discussion and 3 case reports. An enlarged spleen, surgically removed, proved to contain numerous nodules of lymphosarcoma, and the patient died with tumor in lungs, kidneys, mesentery, and retroperitoneum. The lesions found at autopsy are regarded as metastatic. The second patient had a large cyst in the spleen, 12 cm. across; references are given to reviews of this condition. The third patient did not have a tumor, but large infarcts provided a nodular surface leading to an erroneous diagnosis and resection. Illustrations.—E. E. S.

ADRENAL

Tumors of the Adrenal Cortex. A Procedure Whereby the Post-Operative Prognosis May Be Improved. NISSEN, R. [New York, N. Y.] *Exper. Med. & Surg.*, **1**:309-313. 1943.

After removal of a large cortical tumor (670 gm.) of the left adrenal gland from a 54 year old woman, 2 small pieces of the tumor tissue were implanted into the subcutaneous fat close to the incision, in the hope that they would secrete sufficient adrenal cortex hormone to counteract the expected, dangerous, postoperative adrenal insufficiency. The patient suffered from adrenal insufficiency symptoms during the first 2 days after operation, despite the implantation procedure mentioned above and the liberal injection of adrenal cortex hormone preparations. However, improvement began on the third day, and the patient was discharged in less than a month. Masculinization symptoms disappeared within 3 months. Urinary analyses made by E. J. Baumann showed 58 mgm. α- and 16.7 mgm. β-ketosteroids daily before operation, and 0.7 and 0 mgm. respectively of these compounds after operation [how long after is not stated]. The tumor transplants did not diminish in size. They were removed 1 year after operation because the histological report on the original tumor did not exclude the possibility of malignancy. The excised transplants resembled the original tumor morphologically. It is probable that the transplants supplied hormone from the second day after grafting, and supported the patient until the opposite adrenal gland recovered from the compensatory atrophy that had been induced by the excessive hormone-production of the tumor-bearing gland. The choice of a readily accessible site for implantation of such grafts permits excision should the graft become malignant. Photomicrographs.—M. H. P.

Pheochromocytoma of the Adrenal Associated with Persistent Hypertension; Case Report. THORN, G. W., HINDLE, J. A., and SANDMEYER, J. A. [Peter Bent Brigham Hosp., and Harvard Med. Sch., Boston, Mass.] *Ann. Int. Med.*, **21**:122-130. 1944.

Diagnostic and therapeutic implications are discussed.—J. G. K.

Cushing's Syndrome with Possible Pheochromocytoma. Report of a Case. LECOMPTE, P. M. [Fairfield State Hosp., Newtown, Conn., and Yale Univ. Sch. of Med., New Haven, Conn.] *Am. J. Path.*, **20**:689-707. 1944.

A case of Cushing's syndrome associated with an adrenal tumor of uncertain histogenesis, possibly a pheochromocytoma, is described. A high content of 17-ketosteroids was found in a single sample of postmortem urine, but no significant amount of cortin was demonstrated in the neoplastic tissue. Application of the usual histological criteria as well as more refined technics did not yield a clear-cut answer concerning the origin of this neoplasm. Careful study by histochemical methods and by assay of fresh tissue for epinephrine and steroid hormones may be expected to give more definite information than routine histological methods in the investigation of adrenal tumors associated with Cushing's syndrome.—Author's summary.

Adrenal Medullary Tumor (Pheochromocytoma) and Diabetes Mellitus; Disappearance of Diabetes after Removal of the Tumor. DUNCAN, L. E., JR., SEMANS, J. H., and HOWARD, J. E. [Johns Hopkins Univ. and Hosp., Baltimore, Md.] *Ann. Int. Med.*, **20**:815-821. 1944.

A case report.—J. G. K.

CANCER CONTROL AND PUBLIC HEALTH

Cancer Prevention. BOWERS, D. D. [Indianapolis, Ind.] *J. Indiana M. A.*, **37**:169-171. 1944.

The article is chiefly a general discussion of the importance of proper care of precancerous lesions. To avoid

development of breast cancer, attention must be paid to chronic cystic mastitis, and practices that produce stagnation of milk are to be avoided. Many forms of irritants are mentioned as contributing to the development of carcinoma of skin, mouth, and stomach. Rectal papillomas may precede malignant growth in this region, and cervical lacerations seem to predispose to cervical cancer. Emphasis is placed on the value of cleanliness, which is regarded as the best single preventive of those cancers that can be avoided.—E. E. S.

Cancer Control and the Doctor. Lehman, E. P. [Charlottesville, Va.] *Virginia M. Monthly,* **71**:395-396. 1944.

As the campaign of lay education becomes increasingly effective, the doctor's alertness must become intensified.—M. E. H.

A County Cancer Program. Neff, J. L. [New York, N. Y.] *New York State J. Med.,* **44**:519-523. 1944.

A brief description of the activities of the Nassau County Cancer Committee, starting with its organization in 1928. The educational program is planned to include not only the general public but also the practicing physician.—J. L. M.

"Early Cancer is Curable." A Study Outline of Cancer Information. Stovall, W. D. [State Lab. of Hyg., Madison, Wis.] *Wisconsin M. J.,* **43**:340. 1944.

Abstract of a study outline for high school students prepared by the Women's Field Army for the education in cancer of the coming generation.—M. E. H.

Cancer Talks before Lay Groups. Lattimore, J. L. [Topeka, Kans.] *J. Kansas M. Soc.,* **45**:74-75. 1944.

Suggestions concerning subject matter and precautions to be taken by the speaker.—C. W.

STATISTICS

Vital Statistics of 1943. Stocks, P. [Gen. Reg. Off., London, England] *Lancet,* **247**:65-67. 1944.

In England and Wales "Cancer deaths increased from 70,419 in 1942 to 72,158 in 1943. The standardized rate is not yet known, but from 1941 to 1942 an increase of 1197 deaths was accompanied by a fall in the standardized rate from 979 to 977 per million and it is unlikely that the increase of 1739 deaths in 1943 will imply any rise in the rate, being such as might be anticipated from the increasing numbers of persons alive at the ages most liable to death from cancer."—E. L. K.

The Measurement of Morbidity. Stocks, P. [Gen. Reg. Off., London, England] *Proc. Roy. Soc. Med.,* **37**:593-608. 1944.

The methods by which a measurement of morbidity may be attempted differ according to the more acute and transient, or the more lasting character of the disease considered. The author, after treating the first of these two subjects in some detail, states as follows a scheme under which the statistics required for the study of cancer in man might be obtained:

"The statistical work of Derrick, McKinlay, and others on generation mortality suggests that the changes in the average environment to which children born in successive periods of time are exposed in their early years tend to impress themselves on subsequent rates of dying throughout life. The Registrar-General has called attention to progressive movements of the age of maximal death-rate from cancer and some other chronic diseases, and such changes in the age-mortality curve may reflect not what is happening to the disease now but what happened several decades ago when the pathological process was commencing. The conquest of acute disease is likely to continue with increasing rapidity and consequently chronic degenerative diseases will assume a greater importance in the general morbidity picture. It is improbable that such conditions will respond greatly to chemotherapy; it is more likely that we shall have to go back to their beginnings and try to remove or counteract the irritant causes which, when unchecked, eventually produce them. Before we can identify these causes it may be necessary to study by statistical methods the continuous health histories of very large numbers of individuals of all occupations and classes, and in this the methods of morbidity recording I have mentioned will not help us. Cross sections of the population alone, no matter how complete and how frequent, can give no complete answers to these problems of chronic disease. They need to be supplemented by individual health histories extending over long periods of time. . . . .

"The ultimate aim is to keep for every individual a record of every event of health significance from the time of conception to death, and to establish a system by means of which a person does not cease to exist statistically when he removes to another administrative area. The building up on a national scale of individual health histories is no new idea—it has been in our minds at the General Register Office since the time of Farr. Not until now, however, have we had the administrative machinery for it ready at hand and a public opinion educated to the point of being ready to welcome it. In my opinion it should be started forthwith for every newborn child, with, as first objective, the keeping of continuous records of every event directly or indirectly affecting the child's health up to the age of 15. We may anticipate that before the children now being born have reached that age a National Health Service will have been developed which could continue to take care of their records throughout life."

(The references to Derrick and McKinlay are to Kermack, W. O., McKendrick, A. G., and McKinlay, P. L. *Lancet,* **226**:698. 1934; and Derrick, V. P. A. *J. Inst. Actuaries,* **58**:117. 1927).—E. L. K.

Correction

Cancer Research, **5**:56. January, 1945. The last abstract in column 2 should begin: "The esterase activity of numerous normal and neoplastic tissues against methyl butyrate was determined. The extracts of the tissues . . ."